Assessment of the prevalence of periodontal pathogens in the plaque and gingiva of patients with fixed and removable orthodontic appliance

Type of manuscript: Research article
Running Title: Periodontal pathogens in fixed and removable orthodontic appliance

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ABSTRACT:
AIM:
To assess the prevalence of periodontal pathogens in patients with fixed and removable orthodontic appliance.

INTRODUCTION:
Fixed and removable orthodontic appliances may complicate an optimal oral hygiene, and this may result in accumulation of dental plaque and gingival inflammation. Evidence indicates the gram-negative obligate anaerobe Porphyromonas gingivalis and A.actinomycetecomitans as putative periodontal pathogens in subgingival dental plaque. P. gingivalis plays an important role in the onset and progression of periodontal diseases, and it is implicated as an indicator of periodontal disease.
The aim of this study was to evaluate the occurrence of periodontal pathogens in patients with clinical manifestation of plaque-associated gingivitis treated with fixed and removable orthodontic appliances.

MATERIALS AND METHODS:
The study was done using convenience sampling of 10 patients undergoing orthodontic treatment from the Orthodontics Department of Saveetha Dental College, Chennai, out of whom 5 were undergoing fixed orthodontic treatment and 5 were wearing removable orthodontic appliances. The study compared the growth of two periodontal pathogens, Porphyromonas gingivalis and Actinobacillus actinomycetecomitans in the plaque deposits collected during the orthodontic treatment. Subgingival plaque samples were collected by inserting a sterile dental curette into the bottom of the gingival crevice during the clinical examination. The samples were collected from the labial-medial and labial-distal surfaces of teeth 31, 32, 41, and 42. The samples from each tooth were pooled into an Eppendorf tube containing 1 mL of sterile saline and stored immediately at -70 degree Centigrade. Analysis was carried out by Real-time PCR.

RESULT:
Quantification of organisms, Porphyromonas gingivalis and Actinobacillus actinomycetecomitants, have been done for the samples F1,F2,F3,F4,F5 and RA1, RA2, RA3, RA4, RA5 by quantitative PCR (qPCR). It has been found that sample F2 and RA4 has a maximum count of 4178.61943 and 565.56 per ml respectively of P.gingivalis.
It has also been found that sample F2 and RA5 has a maximum count of 3821.7132 and 231.59 per ml of A.actinomycetecomitans respectively.
CONCLUSION:
To create awareness about the occurrence of plaque-associated gingivitis due to periodontal pathogens in patients treated with fixed and removable appliances.

KEYWORDS: Fixed orthodontic treatment, Removable appliance, Periodontal examination, Porphyromonas gingivalis, Actinobacillus actinomycetecomitans, Real-time PCR

INTRODUCTION:

Fixed orthodontic therapy is an effective and common method for treating malocclusions in contemporary orthodontics. Fixed appliances such as brackets, bands, or fixed retentions may complicate an optimal oral hygiene, and this may result in accumulation of dental plaque and gingival inflammation[1,2].

One of the common side effects during orthodontic therapy are gingivitis and periodontitis. It is still unclear whether the periodontal changes in orthodontic therapy could be reversible after the removal of appliances. Most studies indicated that gingival changes were only temporary and could be reversible[3], while a few of researches reported a significantly clinical attachment loss during orthodontic therapy[4-6]. A prospective study discovered that orthodontic accessories had a negative impact on periodontal parameters, moreover these changes were only partially reversible post therapy[5]. It has been recognized that anaerobic microorganisms in the subgingival plaque are the key etiologic factors in the initiation and progression of gingivitis and periodontitis[7]. Recent advancements in the periodontal research field supported the theory that periodontal diseases are resulted from a rupture of the dynamic balance between the relative abundance of periodontopathogens and host defence system[8] .Positive associations between periodontal diseases and several pathogens have been reported, including A. actinomycetemcomitans and P. gingivalis[9-11].

Ample evidence indicates that gram-negative obligate anaerobe Porphyromonas gingivalis plays an important role in the onset and progression of periodontal diseases, and it is implicated as an indicator of periodontal disease[12-16]. Gingival changes and oral bacteria in plaque have been studied during orthodontic treatment in adolescents and young adults[17-19]. Previous researches revealed that P. gingivalis has been categorized as the "red complex" species, which are related to the severity of periodontitis, while A. actinomycetemcomitans is categorized as secondary harmful species involved in periodontitis[20-23]. Additionally, current evidence suggested orthodontic appliances could alter the equilibrium of the microorganism ecosystem, and increased the potential for pathogenicity within the microbial ecosystem[24, 25]. It is crucial to understand the composition and changes of periodontopathogens during orthodontic therapy in order to avoid potentially irreversible injuries caused by orthodontic appliances.

Socransky et al. showed that the presence of fixed orthodontic appliances encouraged the growth of periodontopathic bacteria species such as Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans. Nevertheless, the use of alternative removable orthodontic appliances may allow patients to maintain an adequate oral hygiene and reduce the risk for such negative dental and periodontal complication[27-30].

There is little information on the microbiological evaluation of the subgingival pathogenic microflora via real-time PCR analyses which may be a suitable method to estimate the risk for periodontal disease[31-33].

In this study, we quantify subgingival pathogens of A. actinomycetemcomitans, P. gingivalis with the real-time PCR and tested the clinical parameters in adolescents during orthodontic treatment to assess whether the microbial and periodontal parameters are different between removable and fixed orthodontic appliances.

MATERIALS AND METHODS:

Polymerase Chain Reaction (PCR)

The 3 steps of PCR are repeated for about 30 to 40 times in an automated thermal cycler, which heat and colds the reaction mixture in the tube in a very short time. This result in exponential increase accumulation of the specific DNA fragments.

Quantification methods:

Relative quantification

Relative quantification was used in this study as it was needed to compare changes in multiple samples which varied in quality and quantity.

Relative ratio = concentration of target / concentration of reference

Primer Details:

Organisms Name	Primer Sequences (5'-3')
Porphyromonas gingivalis	FP: GCGTAGGTTGTTCGGTAAGT RP: CATACTGCGACTGACACTGAA
Actinobacillus actinomycetemcomitans	FP : GCCCGAAGCTAACGTGATAA RP: CTTCGGATGTCAAGAGTAGGTAAG

Procedure

1. Take out the samples and all the stocks (from -20°C) needed for Real Time PCR, keep them on ice and allow them to thaw.
2. PCR reaction are set as shown in the Table 1
3. If there are many samples to be analyzed, it is advisable to make cocktail for as many numbers of samples. Cocktail can be prepared with one or two extra volumes so that shortage of reaction mix can be avoided due to pipetting error.
4. The SYB Green I Master mix is light sensitive.
5. The mixture of SYB Green I Master mix, water and primer was mixed, vortexed and spun for few seconds.
6. This reaction mix was loaded onto the Real time PCR plate.
7. Then the respective sample was added to the plate.

8. The plate is spun for few seconds.
9. NTC is mandatory in real time experiments.
10. qPCR was set according to the program given below (Table.2)

Components	Volume (µl)
Sample	5.0
SYB Green I Master mix (2X)	10
Sterilized water	5
Total Volume	20

Table 1: PCR reaction Mixture

qPCR PROGRAM

Step	Temperature	Temperature	No. of cycles
Initial Denaturation	95°C	5 min	1
Denaturation	95°C	20 sec	40
Annealing	58°C	20 sec	
Extension	72°C	20 sec	
MC	95°C- 60°C - 95°C	1 min	1
Cooling	40°C	1min	1

Table 2: qPCR cyclic conditions

RESULTS:

Porphyromonas gingivalis

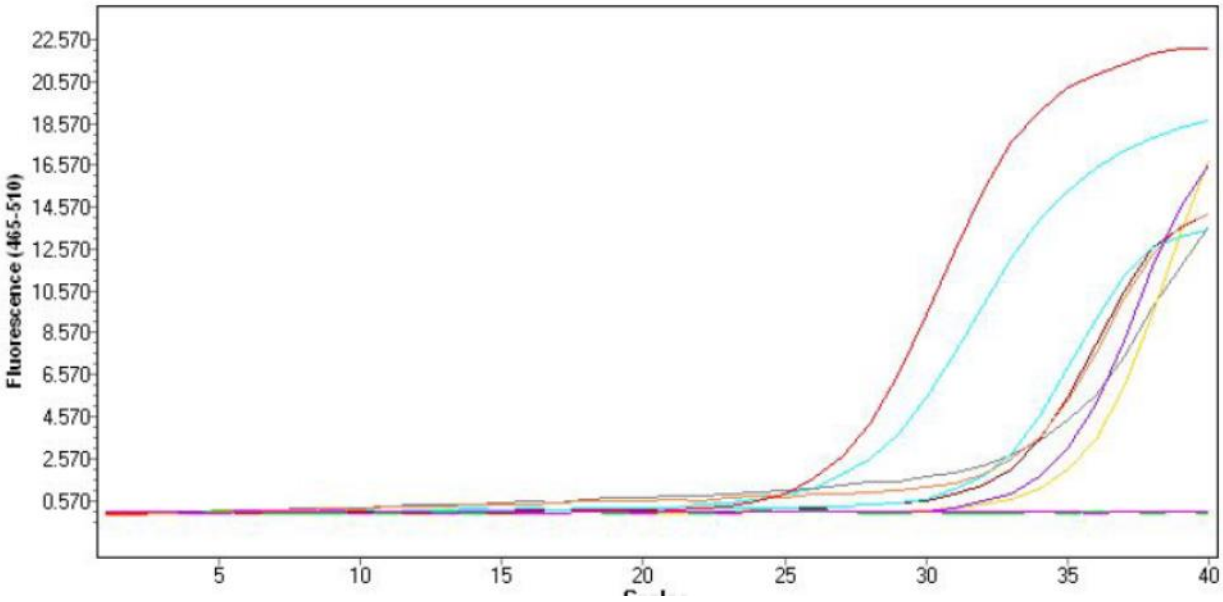

FIG:1 Porphyromonas gingivalis

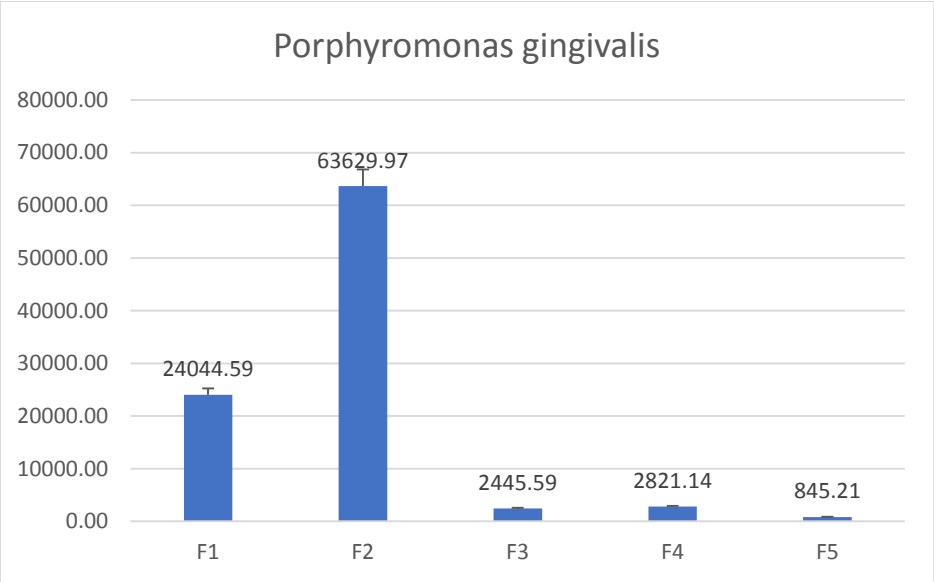

FIG 2: Porphyromonas gingivalis count per mL solution in patients undergoing fixed orthodontic treatment

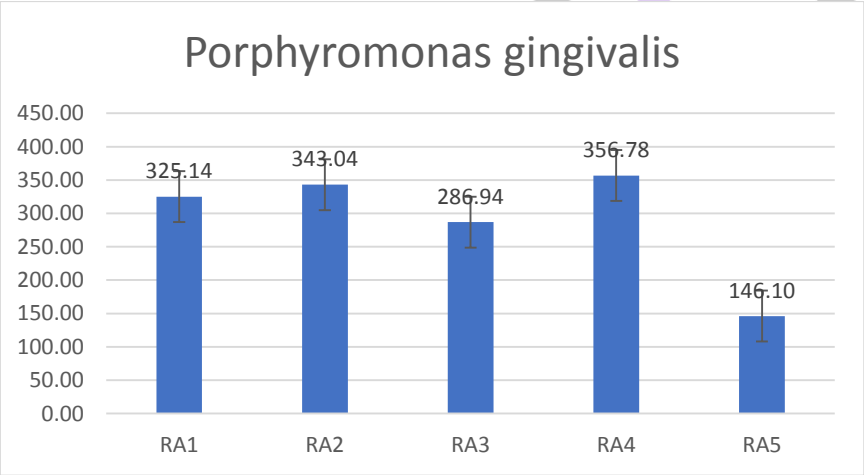

FIG 3: Porphyromonas gingivalis count per mL solution in patients undergoing removable orthodontic treatment.

Actinobacillus actinomycetemcomitans

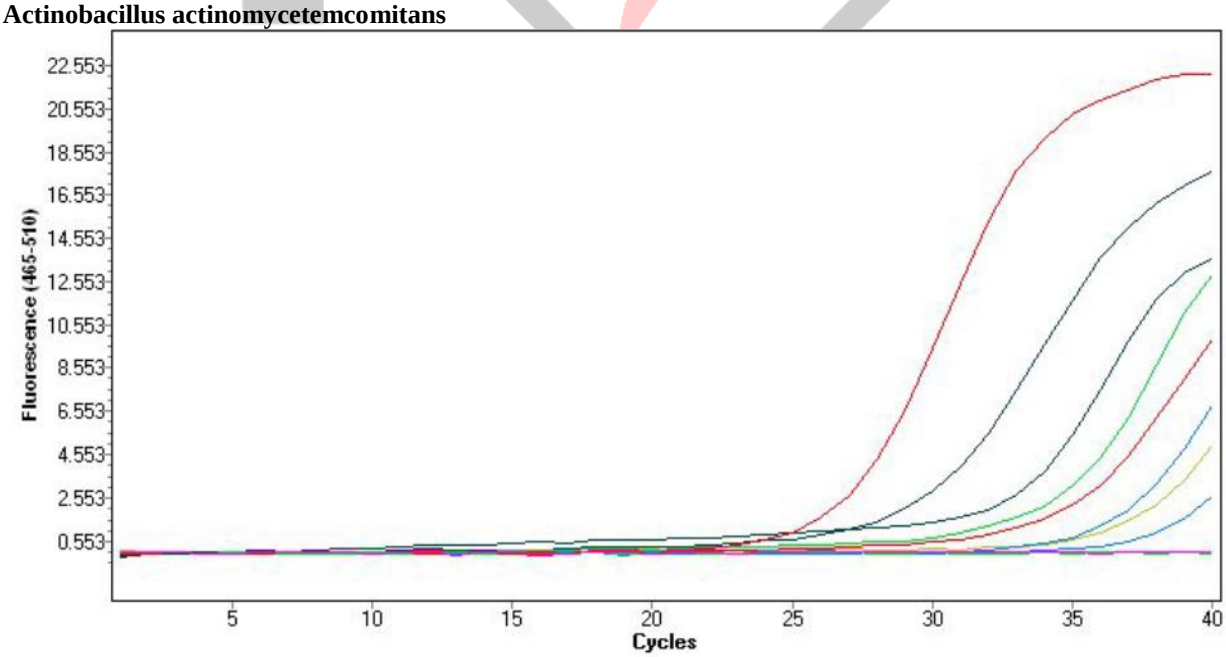

FIG 4: Actinobacillus actinomycetemcomitans

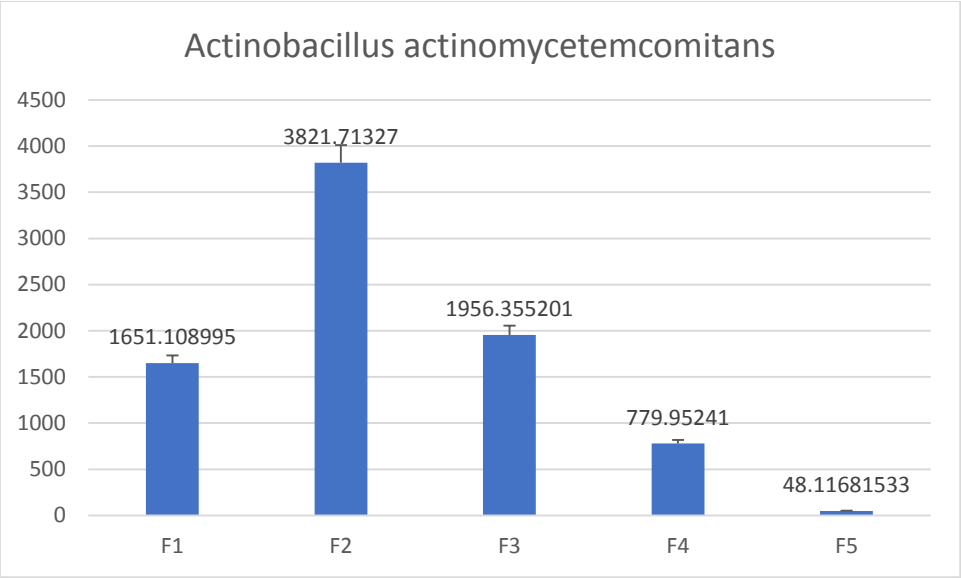

FIG 5: Actinobacillus actinomycetecomitans Count per ml of the solution in patients undergoing fixed orthodontic treatment

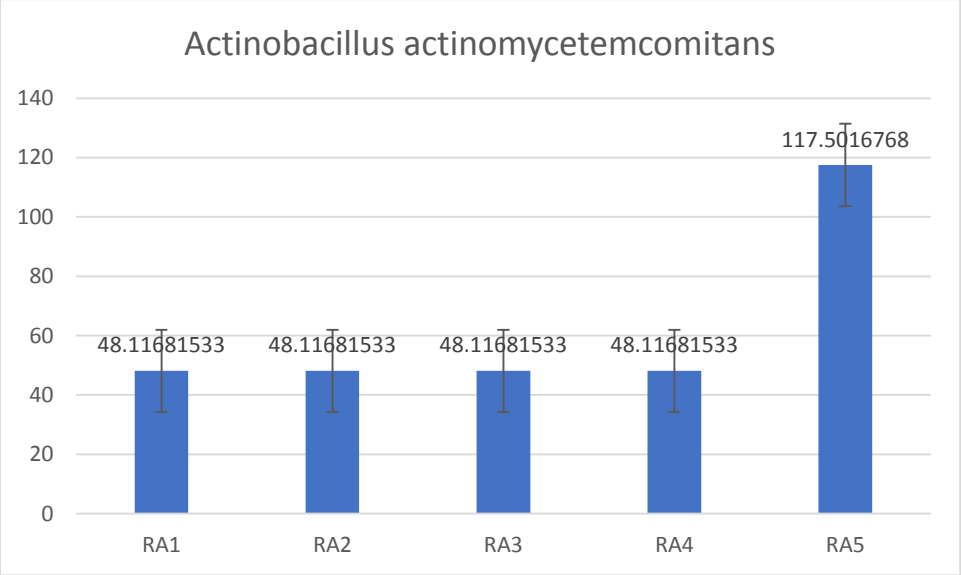

FIG 6: Actinobacillus actinomycetecomitans Count per ml of the solution in patients undergoing removable orthodontic treatment

TABLE 3: Count per mL of P. gingivalis and A. actinomycetecomitans in Fixed appliance patients

Samples	F1	F2	F3	F4	F5
Porphyromonas gingivalis	24044.59	63629.97	2445.59	2821.14	845.21
Actinobacillus actinomycetecomitans	1651.108995	3821.71327	1956.355201	779.95241	48.11681533

TABLE 4: Count per mL of P. gingivalis and A. actinomycetecomitans in Removable appliance patients

Samples	RA1	RA2	RA3	RA4	RA5
Porphyromonas gingivalis	325.14	343.04	286.94	356.78	146.10
Actinobacillus actinomycetecomitans	48.11681533	48.11681533	48.11681533	48.11681533	117.5016768

Quantification of organisms Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans has been done for the samples F1,F2,F3,F4,F5 and RA1,RA2,RA3,RA4,RA5 by quantitative PCR (qPCR).

Porphyromonas gingivalis
It has been found that sample F2 and RA4 has a maximum count of 4178.61943 and 565.56 per ml respectively. (Fig 1,2 and 3)

Actinobacillus actinomycetecomitans
It has been found that sample F2 and RA5 has a maximum count of 3821.7132 and 231.59 per ml respectively. (Fig 4,5 and 6)

DISCUSSION:

The role of microorganisms in the aetiopathogenesis of individual periodontal diseases has been discussed in the literature for many years[53]. This study compared the prevalence of two periodontal pathogens, Porphyromonas gingivalis and Aggregatibacter actinomycetecomitans in patients undergoing fixed and removable orthodontic treatment. P.gingivalis is an obligate anaerobe while A.actinomycetecomitans is a facultative anaerobe with both pathogens playing a significant role in the onset of periodontitis. The results of our study was done using Real-time PCR analysis on the supragingival plaque samples of the study participants. It was found in our study that the growth of the periodontal pathogens P.gingivalis and A.actinomycetecomitans was greater in the samples of patients undergoing fixed orthodontic treatment as compared to patients undergoing removable orthodontic treatment. This is in accordance with previous studies which show Porphyromonas gingivalis and A.actinomycetecomitans have the capacity to penetrate into the buccal epithelial cells in the oral cavity. The released buccal epithelial cells can participate in the transmission of periodontal pathogens among individual localities in the same individual or among several individuals[34,50]. Leung et al. reported that the capacity of bacteria, especially
Actinobacillus actinomycetemcomitans to invade buccal epithelial cells increased after fixing the orthodontic appliance[18]; a probable reason being physical damage of cells by individual components of the orthodontic appliance. It was further found in our study that there was greater prevalence of P.gingivalis than A.actinomycetecomitans in both the fixed appliance as well as removable appliance patients. This is consistent with a study by Baehni et al, according to which P.gingivalis was associated with destructive periodontitis[35].

According to previous studies, authors have demonstrated that the occurrence of bacteria in supragingival and subgingival plaque is similar and supragingival plaque can thus serve as a reservoir for bacteria that can subsequently invade the subgingival spaces[36]. The assessment of occurrence of the individual pathogens confirmed the presence of Porphyromonas gingivalis only in the periodontitis patients. Zadeh et al.suggested that destruction of the periodontium induced by Actinobacillus actinomycetemcomitans (A.a.) is caused by the interaction between this pathogen and immune response of the host[37]. Its presence may be considered a risk factor for the development of periodontopathies. Okada et al. analysed the occurrence of Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis in a study with 104 participants concluding that a higher frequency of pathogens studied in the examined set was related to the presence of the fixed orthodontic appliance[38]. The increased pathogenicity of the dental plaque and the concomitant periodontal changes during orthodontic treatment have been described by several authors (Petti
et al., 1997; Naranjo et al., 2006; van Gastel et al., 2008). Several studies showed that the frequencies and counts of periodontopathogens significantly decreased after appliances removal[40-45]. Kim et al, stated that P.gingivalis dramatically decreased immediately after appliances removal, but A.actinomycetemcomitans remained unchanged[45] . This is due to the fact that the gingival enlargement induced by orthodontic appliances might provide a favorable environment for the colonization and maturation of anaerobic bacteria, and favors a qualitative shift from a predominance of aerobic bacteria to more putative anaerobic periodontal pathogens[19,46]. Therefore, removing the orthodontic appliances eliminates their plaque-retentive effect, which might make practicing good oral hygiene easier[19]. Additionally, this discrepancy of nonsignificant difference in the count of A. actinomycetemcomitans as opposed to significant differences in the count of P. gingivalis may be ascribed to the fact that A. actinomycetemcomitans is a gram-negative facultative anaerobe, while P. gingivalis is an obligate anaerobes, the growth of latter are easily to be stimulated by the ecologic environment induced by gingival enlargement.
Only a few studies have dealt with the effect of a fixed orthodontic appliance on the oral bacterial flora. Lee et al. reported that there were no differences in the frequency of occurrence of Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis compared to the control group of adult patients with gingivitis and without the orthodontic appliance[47]. Conversely, Paolantonio et al. proved that Actinobacillus actinomycetemcomitans colonised subgingival plaque only on teeth with an attached fixed orthodontic appliance[17]. Armitage stated that periodontally healthy subjects carry putative periodontal pathogens as part of the normal oral plaque because these bacteria are detected at low numbers in periodontally healthy individuals[48,49] . These microorganisms may be opportunistic pathogens, the levels of which increase to a critical threshold to induce periodontal tissues destruction. This implies that an increased number of periodotopathogens, rather than frequency, might be an important determinant in the development of periodontal inflammation. Thus, it is reasonable to focus on the numbers of periodontopathogens to prevent periodontal diseases in orthodontic patients.

CONCLUSION:
The growth of P. gingivalis and A.actinomycetecomitans were found to be significantly more abundant in fixed appliances when compared to removable appliances. This study quantified the subgingival pathogens as there is no extensive quantification done in previous studies.
In present times the role of periodontal pathogens in the origin and development of general diseases, such as cardiovascular diseases, cerebral vascular diseases, and low birth weight in infants, has been considered more and more frequently. When performing various interventions in the oral cavity (e.g. in professional hygienic treatment), it is necessary to keep in mind that periodontal pathogens

may penetrate into the patient's system. Prevention of any such diseases can be brought about by maintenance of proper oral hygiene in patients with fixed orthodontic appliance as well as suitable antimicrobial measures.

ACKNOWLEDGEMENTS:
We are grateful to the patients for their contribution to the work. We also want to thank Biozone Research Technologies, Chennai, for their assistance in bacteria culture. This work was supported by Saveetha dental College, Chennai.

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