

# Assessment of subgingival microbial changes in plaque before and during the early phase of Orthodontic treatment using the RT-PCR method. (Real-time polymerase chain reaction method).

Dr. Rahamtulla Syed, Dr. Kola. Srikanth Reddy, Dr. Vem Preethi Reddy, Dr. A. Aravindh Mar, Dr. Mallavarupu Haripranithi, Korokoppula Alkeya,

<sup>1</sup>associate Professor Department Of Orthodontics. Care Dental College & Hospital, Guntur, A.P, India Email Address: Sd.Raham@Gmail.Com.

<sup>2</sup>professor Department Of Pedodontics & Preventive Dentistry

Meghana Institute Of Dental Sciences Nizamabad, Telangana State, India

Email Address: Kolasrikanthreddy@Gmail.Com

<sup>3</sup>senior Lecturer. Department Of Pedodontics & Preventive Dentistry.

Malla Reddy Dental College For Women. Mallareddy Viswavidhyapeet Deemed To Be A University. Hyderabad, Telangana State, India

<sup>4</sup>professor. Department Of Periodontics. Meghna Institute Of Dental Science, Nizamabad, Telangana State.

<sup>5</sup>senior Resident. Government Dental College, Hyderabad, Telangana State.

<sup>6</sup>asst.Professor.Meghna Institute Of Dental Science, Nizamabad.

Telangana State, India.

## HOW TO CITE:

Rahamtulla Syed, Kola. Srikanth Reddy, Vem Preethi Reddy, A. Aravindh Mar, Mallavarupu Haripranithi, Korokoppula Alkeya, (2026). Assessment of subgingival microbial changes in plaque before and during the early phase of Orthodontic treatment using the RT-PCR method. (Real-time polymerase chain reaction method). International Journal of Special Education, 41(21s),1467-1476

## ABSTRACT

**Objectives:** Evaluate changes in subgingival microbial communities before and during the levelling and alignment stages of orthodontic treatment using RT-PCR.

**Materials & methods:** This study included 30 orthodontic patients aged 12 to 25. Subgingival microbial samples were obtained from the distobuccal crevices of the upper and lower right and left central incisors, as well as from the mesiobuccal crevices of the upper and lower right and left first molars. Samples were collected at 3 times points: baseline, before & during Orthodontic appliance placement after one month (T1), two months after placement (T2), and 3 months after placement. (T3). DNA was extracted from the samples, and a 16S rRNA-based RT-PCR method was employed to assess the prevalence of *P.gingivalis*, *T.forsythia* (T.f), and *P.intermedia* (P.i), which are identified as putative periodontal pathogens.

**Results:** Comparing the prevalence of the 3 periodontal pathogens, *P.gingivalis*, *T.forsythia*, and *P. intermedia*, at T1, T2, and T3 showed significant differences. Notably, there was an

**COPYRIGHT STATEMENT:** increase in the levels of these pathogens in the subgingival plaque after 3 months of Orthodontic treatment.  
Copyright: © 2026 Authors.

Open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

**Conclusion:** Orthodontic appliance placement leads to an increased prevalence of periodontal pathogens during the early stages of treatment, potentially adversely affecting periodontal health.

**Keywords:** Gingivitis, Orthodontic treatment, RT-PCR, Periodontal pathogens.

## 1. Introduction

Malocclusion is a prevalent oral and maxillofacial condition, and fixed orthodontic therapy is commonly recommended to achieve an aesthetically pleasing dentofacial appearance and improved occlusion. [1,2,3,4,5] The keystone hypotheses holds that certain low-abundance Microbial pathogens can orchestrate inflammatory diseases by remodelling a normally benign microbiota into a dysbiotic one.[6] The alternative hypothesis argues that specific microorganisms are responsible for causing distinct types of periodontal diseases.[7] It is essential to monitor changes in the oral environment of patients undergoing orthodontic treatment as well as other factors that may influence the development or progression of periodontal diseases.

However, the techniques such as routine bacterial culture, immune assays, biochemical tests, and hybridization methods require bacterial cultures, making them both laborious and time-consuming.[8] There has been a strong emphasis on RT-PCR (Applied biosystem 7900HT fast) a culture-independent method with higher sensitivity and specificity, especially for detecting anaerobic bacteria. However, this method still faces challenges when it comes to quantification. Recently, real-time polymerase chain reaction (RT-PCR) has been developed to quantify DNA by continuously measuring the accumulation of RT-PCR products throughout the process. This method provides a simple, fast, and quantitative way to detect microorganisms in clinical samples that would otherwise be undetectable using conventional culture techniques. [9,10]

This study focussed on identifying and quantifying periodontal pathogens, namely (P.g.) (T.f) and (P.i), in subgingival plaque samples collected from patients undergoing orthodontic treatment. It employed RT-PCR, a recent diagnostic advancement, to detect even low levels of these pathogens in the samples.

### Aims of the study;

To evaluate the changes in sub-lingival microbiology of plaque before and during the early stages of orthodontic treatment using Real-time polymerase chain reaction (RT-PCR).

### Objectives;

1. To assess the prevalence of periodontal pathogens in subgingival plaque before the commencement of orthodontic treatment.
2. To evaluate the prevalence of periodontal pathogens in subgingival plaque before and during the initial phase of orthodontic treatment.
3. To compare the microbiological changes in subgingival plaque before and during the initial period of orthodontic treatment.

## 2.2 Materials & methods;

### 2.1 The study obtained approval from the institutional Ethics Committee.

For sample size calculation, we considered the study by Sang Ho Kim et al (2012). Hence, the association of orthodontic appliances with the presence of periodontopathogens was determined using the chi-square test, as we had 2 categorical variables. One being the number of pathogens according to the index scores and the other the time intervals at T1, T2 and T3. Thus, the effect size or Cohen's w was taken as 0.3

(medium). Significance ( $\alpha$ ) level at 0.05 or 5%. Power  $1-\beta = 80\%$  or 0.80. So, using these values and considering the study of Sang Ho kim et al the sample size arrived at was 30.

This method involved 30 subjects (17 Females & 13 Males) aged 12 to 25, who were randomly selected at the beginning of their orthodontic treatment in the department.

#### **Inclusion criteria;**

1. Subjects were chosen from the age range of 12 to 25 years.
2. General health; subjects had no history of medication that could impact their gingival or Periodontal health.
3. Subjects had not undergone periodontal treatment in the past 6 months, exhibited healthy gingiva with a probing depth of less than 4 mm, and had both plaque and gingival indices below 1.
4. Orthodontic appliances; Subjects both upper & lower arches to be treated with fixed labial appliance.

#### **Exclusion criteria;**

1. Subjects with systemic diseases were excluded.
2. Subjects who had orthodontic treatment initiated.
3. Subjects who had periodontal treatment in prior six months.

4. Subjects who had missing teeth or other premolars extracted for orthodontic reasons were excluded.

The subjects were split into 2 groups;

**For group A;** Sub gingival plaque samples were collected before placement of orthodontic appliance at 3 different time points; one month (T1), two month (T2) and Three months (T3).

**Group B;** Sub gingival plaque samples were collected after removal of orthodontic appliance at 3 different time points; one month (T1), two months (T2) and Three months (T3).

#### **Patients In Group A:**

were advised to improve their oral hygiene by brushing more frequently and for longer periods, but without the use of Antibacterial mouthwashes.

#### **Patients in Group B;**

Were advised to improve their oral hygiene by brushing more frequently and for longer periods, but without the use of Antibacterial mouthwashes.

Patients in Group B were also instructed to visit their dentist after appliance removal for plaque and calculus removal, followed by a tooth polishing procedure.

Participants were asked to avoid eating, drinking, and oral hygiene practices for 3 hours before subgingival plaque collection on the sampling day. **(Fig1)**



**Figure 1; Subgingival plaque sampling after careful removal of supragingival plaque.**

Subgingival sampling procedure involves collecting bacterial plaque or fluid from beneath the gumline inside a periodontal pocket using sterile paper points, specialized endodontic /absorbent strips or miniature curettes for microbiological analysis.

Subgingival plaque samples were obtained from the distobuccal crevices of the upper and lower

right and left central incisors, and from the mesiobuccal crevices of the upper and lower right and left first molars at three different time points. At baseline, before placement of orthodontic appliance (T1,T2,T3),one month (T1),Two months (T2), Three months (T3) after orthodontic appliance placement.(Fig2)



**Figure 2; Subgingival plaque sampling after careful removal of supragingival plaque.**

#### **PCR-based microbiological Analysis;**

PCR-based microbial analysis is a highly sensitive method it can detect very low amounts of target DNA or RNA ,amplify specific pathogen sequences in vitro, and yield results much faster than traditional culture techniques.

Cotton rolls were used to isolate the sampling sites, which were then dried with a gentle air stream. Sterile paper points were inserted into the subgingival space to a depth of approximately 1 micrometre and left in place for 15 seconds the paper points were then transferred to a sterile tube and stored at -80°C. Bacterial DNA was examined using the boiling method described by Gebara et al; [11] Real-time PCR was then employed to detect the presence of major periodontal pathogens, including *P.gingivalis* (P.g), *Tannerella.forsythia* (T.f), and *Prevotella intermedia* (P.i).

The primer sequences for the 16 s r RNA gene of the analyzed bacteria are listed in Table 1. The

PCR mixture, totaling 25 micro litre, contained 1 micro litre each of 5 micrometre upstream and downstream primers, 5 micro litre of 10x PCR buffer, 1 microlitre of 0.2 Mm Dntp mix, 1 unit of Taq polymerase, 3 micro litre of bacterial DNA, and distilled water to make up the remaining volume to 25 micro litre. The Amplification process involved 35 cycles in a Thermal cycle (PCR express, Hybrid, USA) Starting with an initial denaturation step at 94°C for 3 minutes. Each of the 35 Amplification cycles included denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72 °C for 3 minutes the final extension step was performed at 72 °C for 7 minutes. (Fig3)

RT-PCR products were Electrophoresed on an 8% Polyacrylamide gel staining the gel with Ethidium bromide, Bacterial DNA was observed under UV light. (Power station 300 plus, labnet International, Inc).



**Figure 3; Sampling positioned in the stand for storage.**

### **Molecular methodology;**

Real-time quantitative PCR (qPCR) is a molecular method that amplifies and simultaneously measures the amount of a target DNA or RNA molecule.

The core technique relies on fluorescent reporter systems such as DNA-binding dyes or sequence-specific probes to track the reaction progress cycle by cycle.

Key steps in the method;

Sample preparation: Extract pure DNA or convert RNA to complementary DNA (cDNA) using reverse transcriptase.

Reaction setup: Mix the template, specific forward and reverse primers, DNA polymerase, Nucleotides (dNTPS), and a fluorescent reporter like SYBR Green or a TaqMan probe.

Thermal cycling: Run repeated cycles of denaturation (Breathing stands apart), primer annealing (binding), and extension (polymerase building new strands).

Fluorescence detection;

Measure the emitter's light signal at each cycle, which grows brighter as more target product is copied.

### **2.2 Data analysis & Quantification;**

#### **Cycle threshold (ct or cq);**

The fractional cycle number at which the fluorescent signal crosses a set background threshold above the noise.

Inverse relationship: A lower ct value means more starting material was present in the sample, while a higher ct value means less starting material.

Quantification types;

Use absolute quantification via a standard curve of known concentration, or relative quantification (such as the ct method) to compare gene expression changes against a reference control.

### **2.3 Data analysis;**

A chi-square test was used to compare the group's significance was determined with a p-value of  $< 0.005$ . The chi-square test was applied to compare the scores of *P.gingivalis*, *T.forsythia*, and *P.intermedia* across the 3 time periods, as well as the cumulative scores for all three species at these intervals, using SPSS software (version 17.0).

### **3.Results:**

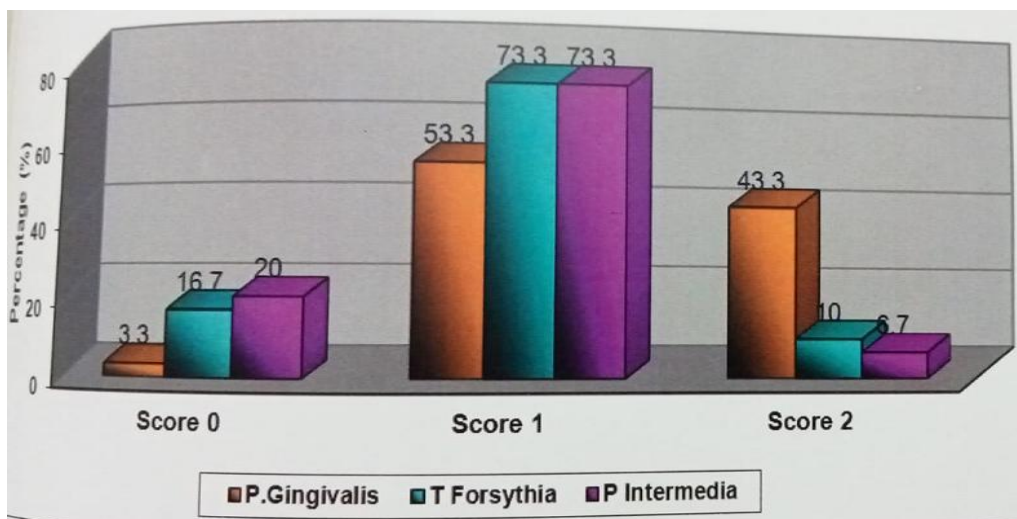
**Table 1** shows the sequence of the primers for the 16 s r RNA gene of the analyzed bacteria.

**Table 2** demonstrated a significant difference in the high scores for *P. gingivalis* and *T.forsythia* at T1, T2, and T3 with a P value of  $< 0.05$ . The comparison of T1 with T2 and T1 with T3 revealed a significant difference for *P.intermedia*, with a P value of  $< 0.05$  in the comparison between T1 and T3 was statically significant. This suggests that the prevalence of periodontal pathogens increases from baseline to 1month &

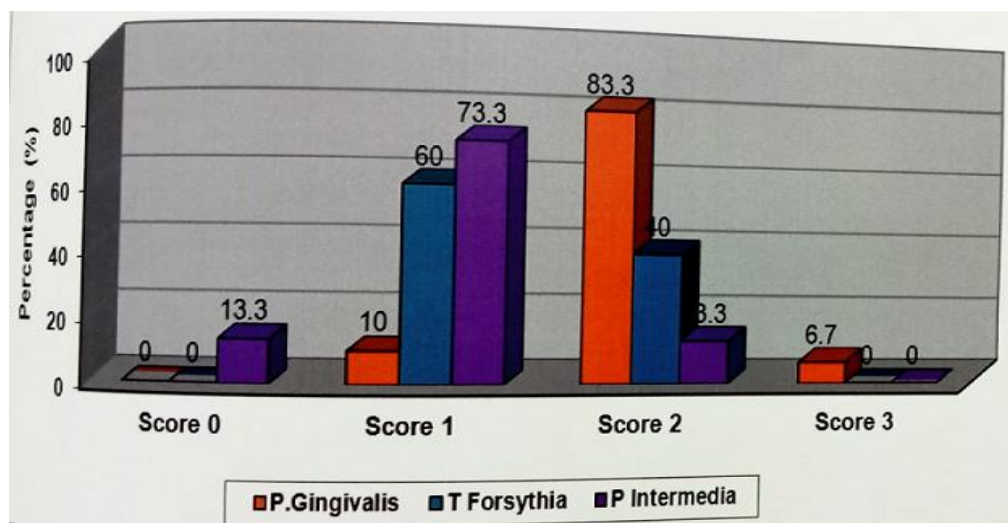
3 months after the start of Orthodontic treatment. (Graph1).

**Table 3** reveals a significant difference in the overall prevalence of *P.gingivalis*, *T.forsythia*, and *P.intermedia* when comparing T1 to T2 and T3 ( $p < 0.05$ ). This suggests an increase in the

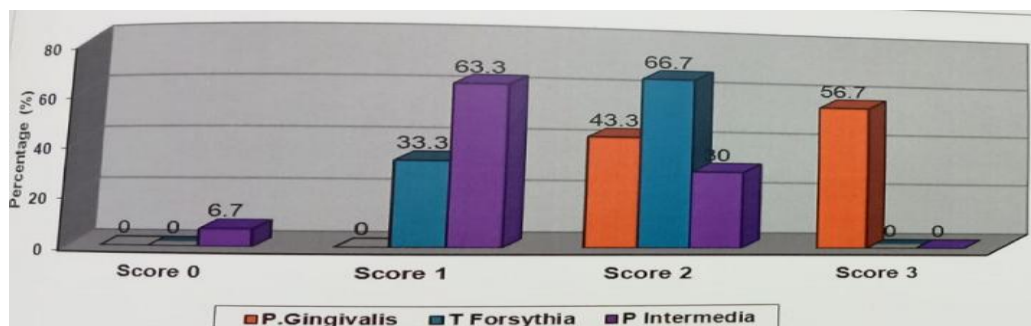
prevalence of these periodontal pathogens from baselines to 1 month and 3 months after the start of orthodontic treatment, although the increase is not statistically significant between T2 and T3. (Graph2).



**Graph 1;** Distribution of three periodontal pathogens with different scores (%) at T1.



**Graph 2;** Distribution of three periodontal pathogens with different scores (%) at T2.



**Graph 3;** Distribution of three Periodontal pathogens with different scores (%) at T3.

**Table 1; PCR primers.**

| Score/time interval | T1 |      | T2 |      | T3 |      | Chi- square test. |                 |                 |
|---------------------|----|------|----|------|----|------|-------------------|-----------------|-----------------|
|                     | N  | %    | N  | %    | N  | %    | T1 vs T2<br>Sig   | T1 vs T3<br>Sig | T2 vs T3<br>Sig |
| Score 2             | 18 | 17.8 | 41 | 40.5 | 42 | 41.5 | .003              | .002            | .913 NS         |

**Table 2; Comparison of the three pathogens with high scores (%) between three different time periods.**

P<0.05, NS –not significant

**Table No. 3: Cumulative scores (%) of the three pathogens at three different time intervals.**

P <0.05, NS –not significant.

#### 4.4 Discussions;

The study indicates significant changes in the levels of P.gingivalis and T.forsythia following

Orthodontic treatment, with scores showing a marked difference from baseline ( $p<0.05$ ). In contrast, P. intermedia was primarily found in deeper periodontal pockets as observed by Lee S.M et al [12]. These findings suggest that environmental changes caused by the treatment may have altered the natural oral

| Specific organisms time interval | T1 |      | T2 |      | T3 |      | Chi-square test |          |          |
|----------------------------------|----|------|----|------|----|------|-----------------|----------|----------|
| P.gingivalis                     | N  | %    | N  | %    | N  | %    | T1 vs T2        | T1 vs T3 | T2 vs T3 |
| T.forsythia                      | 13 | 43.3 | 27 | 90   | 30 | 100  | .027            | .010     | .691 NS  |
| P.intermedia                     | 3  | 10   | 12 | 40   | 20 | 66.7 | .020            | .005     | .564 NS  |
| P.intermedia                     | 2  | 6.7  | 4  | 13.3 | 9  | 30   | .414NS          | .035     | .166 NS  |

microflora. A comparison of pathogen levels from baseline to 3 months post-orthodontic treatment for P.gingivalis, T.forsythia and P.intermedia intermedia demonstrates a significant increase from T1 to T3 ( $p<0.05$ ), with a notable rise in the proportions of the species over this period.

P.gingivalis, T.forsythia and P.inetrmedia are anaerobic, Gram -ve oral bacteria strongly linked to gum diseases, where P.gingivalis and T.forsythia act as primarily red complex pathogens, and P. intermedia contributes heavily to plaque build-up, Gingivalis, and tissue damage.

| Primers Size.                                                                     | Primers Size (bp) |
|-----------------------------------------------------------------------------------|-------------------|
| Universal 16S rDNA 5' AGA GTT TGA TCC TGG CTC AG 3'                               |                   |
| <i>Porphyromonas gingivalis</i> 5' CAA TAC TCG TAT CGC CCG TTA TTC 3'             | 400               |
| <i>Aggregatibacter actinomycetemcomitans</i> 5' CAC TTA AAG GTC CGC CTA CGT GC 3' | 600               |
| <i>Tannerella forsythia</i> 5' GTA GAG CTT ACA CTA TAT CGC AAA CTC CTA 3'         | 840               |
| <i>Prevotella intermedia</i> 5' GTT GCG TGC ACT CAA GTC CGC C 3'                  | 660               |

*P. gingivalis*: A major driver of severe periodontitis that uses toxic enzymes called gingipains to break down tissue and helps form stubborn dental biofilms.

*T. Forsythia*: An anaerobic rod that works alongside *P. gingivalis* to destroy gum tissue and support bone loss around the teeth.

*P. intermedia*; A microbe often found in deep gum pockets that thrives during early gum inflammation, bleeding and pregnancy-related gingivitis.

The rise in pathogens % from baseline to 3 months following Orthodontic with numerous other reports. These findings are consistent with those of KIM S.H et al [14]. Who observed an increase in periodontal pathogens before and during orthodontic treatment. Specifically, *P.gingivalis* rose from 6.7% to 10%, *T.forsythia* from 7.5 % to 25.8% *P.forsythia* from 7.5 % to 25.8%, and *P.intermedia* from 5.0% to 13.3%.

Naranjo A. A et al [15] found a significant increase ( $p < 0.05$ ) in the counts and frequencies of *P.gingivalis*, *T.forsythia*, and *P.intermedia* in sub gingival plaque 3 months after bracket placement. This indicates that orthodontic treatment may affect the presence of periodontal microorganisms. The present study found a significant increase in *T.forsythia* and *P.intermedia* from baseline to 3 months during orthodontic treatment these results align with

Thronberg MJ et al [16] who observed a similar increase in these pathogens from baseline to 6 months during Orthodontic treatment.

This indicates that the initial stages of orthodontic tooth movement may create a more conducive environment for a periodontal pathogen. Several studies [12,13,17,18] have reported elevated levels of *T.forsythia* and *P.intermedia* in periodontal lesions. The present study also found similar results, suggesting that elevated periodontal pathogens might be related to periodontal damage. Further more, other research indicates that orthodontic treatment could be associated with damage to the periodontium.

Additionally, studies have shown that orthodontic treatment may be linked to damage to the Periodontium. Moreover, research indicates that orthodontic treatment can lead to bone loss in the periodontium and a notable reduction in clinical attachment shortly after fixed orthodontic appliance are applied. [19]

Liu H et al [20] Naranjo Jo et al [15] Lee et al [12] have all reported a marked increase in periodontal clinical parameters, including the gingival index, plaque index, bleeding on probing, and periodontal pocket depth, shortly after start of fixed orthodontic treatment, while these studies linked these changes to the presence or absence

of putative periodontal pathogens, none of them have provided a quantitative analysis.

In this study, putative periodontal pathogens demonstrated a moderate to strong expression, reflected by the cycle threshold values. (semi-quantitative analysis) comparison with previous studies suggests an increase in the presence of putative periodontal pathogens, such as *P. gingivalis*, *T.forsythia*, and *P.intermedia*, after the placement of fixed orthodontic appliance. This rise, observed both qualitatively and quantitatively could lead to term periodontal breakdown. The findings indicate that professional oral prophylaxis along with diligent oral hygiene practices is essential for controlling plaque, regular maintenance in shorter intervals is recommended to ensure periodontal health is maintained both during and after orthodontic treatment.

## References;

- Brett Eckley, John Thomas, Richard crout, Peter ngan(2012) .Periodontal and microbiological status of patients undergoing orthodontic therapy. Hong Kong Dent J.9(1);1467-1476.
- Eid H.A ,Assiri H.A.M ,Kandyala R,Togoo R.A,Turakhia V.S(2014) .gingival enlargement in different age groups during fixed orthodontic treatment .Journal of international oral health; 6(1):1467-1476.
- Kloehn JS, Pfeifer JS(1974).The effect of orthodontic treatment on the periodontium angle orthod.44(2):1467-1476.
- Zachrian S,Zachrisson BU(1976).Cause and prevention of injuries to teeth and supporting structures during orthodontic treatment.AMJ Orthod.69(3):1467-1476.
- Zachrisson S,Zachrisson BU(1972).Gingival condition associated with orthodontic treatment. Angle Ortho42(1):1467-1476.
- Richard P.darvean, and Michael A(2012).George hajihengallis, Richard P.darvean, and Michael A.curtiset al .The key stone pathogen hypothesis.NatRevMicrobial.10(10):1467-1476.
- Slots.J,Listgarten MA(1988).Bacteriodes gingivalis,bacteriodes intermedius and Actinobacillus Actinomycetemitans in human periodontal diseases.Jclin Periodontal 15(2):1467-1476.
- MaedaH,FujimotoC,HarubirY,MaedaT,Kokeguchi,S,Petelin,M.Takashiba,S(2003),Quatititative real-time PCR using taqman and SYBR green for Actinobacillus actinomyceteam comitana,porphyromonas gingivalis,prevotella intermedia gene and total bacteria.FEMS immunology & medical microbiology .39(1):1467-1476.
- Boutaya R, Vanwinkehoff AJ, Vandebroucke-granuls CM, Savelkoul PH(2006).The additional value of real time PCR in the quantitative detection of periodontal pathogens.J Clin Periodontal 33(6):1467-1476.
- Nonnemacher C, Dalpke A, Mutters R, Heekgk.(2004).Quantitative detection of periodonto pathogens by real time PCR .JMicrobiolomethod.59(1):1467-1476.
- Gebara EC,Pannuti C, Faria CM,Chehter L,Mayer MP,Lima LA(2014).Prevalence of helicobacter pylori detected by polymerase chain reaction in the oral cavity of periodontitis pateints.Oral Microbial Immunol.19(4):1467-1476.

## 5. Conclusion:

1. Orthodontic appliance placement can raise the levels of periodontal pathogens during the early phase of treatment, potentially harming periodontal tissues.
2. A shift towards a periodontopathogen microflora was observed, with elevated levels of *P.gingivalis*, *T.forsythia*, and *P.intermedia*.
3. Oral hygiene measures should be emphasised during orthodontic treatment to avoid adverse effects on the periodontium.

## Limitations

1. The study did not include a separate control group, as baseline samples served as the control.
2. Periodontal clinical quantification was achieved through the SYBR Green method.

- lee SM,YooSY,Kim HS,Kim KW,Yoon YJ,Lim SH,Shim HY,Kook JK(2005).Prevalence of putative periodontopathogens in sub gingival dental plaque Periodontopathogens in sub gingival dentall plaque from gingivitis lesion in Korean orthodontic patients. J Microbial 43(3):1467-1476.
- Matto J,Saarela M,Alauusua S,Oja V,Jousimies somer H,Asikainen S.(1998)Detection of Porphyromonas gingivalis from saliva by PCR by using a simple processing method.JClin Microbiol .36(1):1467-1476.
- Kim SH,Choi DS,Jang I,Cha BK,Jost-Brinkmann PG,song JS.(2012)Microbiologic changes in sub gingival plaque before and during the early period of orthodontic treatment .Angle Orthod .82(2):1467-1476.
- Narajo AA,Trivino ML,Jaramillo A,Betancourth M,Botero JE.Changes in the sub gingival microbiota and periodontal parameters before and 3 months after bracket placement.AMJ orthodontofacial ortho 2006;130(3):1467-1476.
- Thornberg MJ,Riolo CS,Bayirli B,Riolo ML,Van Turberg EA,Kullbersh R(2009).Periodontal pathogens levels in adolescents before, during and after fixed orthodontic appliances therapy. Am J Ortho facial ortho .135(1):1467-1476.
- Sallum EJ,Nouuuer DF,Klein MI,Goncalves RB,Machion L,Wilson Saalum A,Sallum EA.(2004).Clinical and microbial changes after removal of orthodontic appliances.AMJ Orthod Dentofacial Ortho,126(3):1467-1476.
- Atack NE,Sandy JR,Addy M.(1996).Periodontal and microbilogical changes associated with the placement of orthodontic appliances.Areview.J Periodontal.67(2):1467-1476.
- Van Gastel J,Quirynen M, Teughels W,Coucke W,Carels.(2008). Longitudinal changes in Microbiolgy and clinical periodontal variables after placement of fixed orthodontic appliance.JPeriodontol .79(11):1467-1476.
- Liu H,Sun J,Dong Y,Lu H,Zhou H,Hansen BF,Song (2011).Periodontal health and relative quantity of sub gingival porphyromonas gingivitis during orthodontic treatment. Angle orthod 81(4):1467-1476.