

Estimation And Comparison of Red Complex Bacterial Level in Malocclusion Patients with And Without Fixed Orthodontic Appliances- A Clinical and Microbiological Study

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Abstract

Objective: The objective of this retrospective study was to evaluate the clinical and microbiological effect of molar bands in malocclusion patients with fixed orthodontic appliances and compare it with patients who are not undergoing orthodontic treatment.

Methods: The present retrospective study was carried out on a total number of 30 subjects, who visited Departments of Periodontics and Orthodontics, SRM Kattankulathur Dental College, Potheri. The subjects chosen were patients diagnosed with malocclusion. Group I (ortho treated) includes those who were about to undergo fixed orthodontic therapy and those who have not opted for the same were allocated under group II (control). Clinical parameters (PI, GI, BOP, PPD, CAL) were recorded, pooled subgingival plaque samples were collected for assessment of bacterial load of (P.gingivalis, T. Denticola, T.forsythia) red complex bacteria and subjected to Real time PCR assay. Clinical and microbiological data were collected at baseline, 1 month, 3 month and 6 month intervals from both groups and results were subjected to statistical analysis.

Results: A significant increase of all clinical parameters including PI, GI, BOP, PPD & CAL along with red complex bacterial level were seen in patients with molar bands over the recommended time intervals when compared to those left untreated.

Conclusion: Proper oral hygiene practices and routine periodontal check-up are inevitable during active orthodontic treatment as well as in the presence of malalignment. Since more periodontal disease risk was expressed during fixed orthodontic therapy more attention towards oral hygiene maintenance during active treatment period is essential.

Keywords: Fixed orthodontic appliance, Periodontal pathogens, Real Time - Polymerase Chain Reaction.

Introduction

Fixed orthodontic therapy is an effective and common method for treating malocclusion in modern orthodontics. One of the common demerits during orthodontic therapy is periodontal complication including gingivitis and periodontitis. It has been documented that anaerobic microorganisms in the subgingival plaque are the crucial etiologic factors in the

initiation and progression of gingivitis and periodontitis.

Additionally, current evidence proposes orthodontic appliances could alter the equilibrium of the microbial ecosystem and amplified the potential for pathogenicity within it(1).

The presence of orthodontic band, bracket, wire and other auxiliary factors like misaligned teeth, faulty restorations, fixed crowns, removable prostheses etc create intense challenge for hygiene maintenance and interproximal dental care becomes more challenging especially in the posterior region. Orthodontic molar banded areas promotes the growth of periodontopathogens since it is a major niche for plaque accumulation. It has been assumed that permanent loss of crestal alveolar bone may be the impact of both increased retention of microbial plaque around fixed appliances and higher osteoclastic activity during tooth movement. The aim of this retrospective study was to evaluate the clinical and microbiological effect of molar bands in malocclusion patients with fixed orthodontic appliances and compare it with those patients who are not undergoing orthodontic treatment

Methods

SUBJECT SELECTION:

The present retrospective study was carried out on a total number of 30 subjects, who visited Departments of Periodontics and Orthodontics, SRM Kattankulathur Dental College, Potheri. The subjects chosen were mild gingivitis patients diagnosed with malocclusion. Group I(ortho treated) includes those who were about to undergo fixed orthodontic therapy and those who have not opted for the same were allocated under group II(control). Subjects were chosen as per the guidelines of the Institutional Ethical Committee. Subjects (initial gingivitis patients) of both the sexes were chosen, within the age range of 18 – 30 years. Informed consent was obtained from each subject prior to enrolment in the study. The study proposal was placed before the institutional scientific and ethical committee board and was approved (Ethical clearance number: 1249/IEC/2017) before the commencement of the study. Excluded from the study were users of systemic or subgingival antimicrobials within 3 months before the baseline examination; users of systemic medication for chronic diseases; those who had undergone periodontal treatment within 3 months before the baseline examination; and smokers, pregnant or lactating women, and patients with any systemic disorder that could influence periodontal conditions or response to treatment.

Fifteen subjects who visited the department of Orthodontics those who were about to undergo fixed orthodontic therapy. They were seen just prior to fixation of molar bands and oral hygiene instructions were given. After baseline (just before placement of molar bands), clinical and microbiological evaluation of all individuals was done at 1 – month, 3-month and 6-month intervals of treatment. Fifteen subjects with malocclusion, those who did not undergo orthodontic treatment. Clinical and microbiological evaluation was done of all subjects at similar intervals as that of Group 1. 30 subjects were selected as per feasibility to perform Real time PCR technique since we took 4 samples from each subject.

CLINICAL MEASUREMENT

For two groups, clinical parameters including plaque index (PI), gingival index (GI), bleeding on probing index (BOP) probing pocket depth (PPD) and clinical attachment loss (CAL) were assessed (Figure.1). Measurements were taken at four time periods: Baseline T0 = before placement of appliance, T1 = one month after placement, and T3 = three months after placement and T6 = 6 months after placement. Periodontal examination and sample collection were performed in department of Periodontics.

MICROBIAL SAMPLING

The analysis of the collected plaque is aimed at detecting and quantifying the following microorganisms: *Porphyromonas gingivalis* (P.g.), *Tannerella forsythia* (T.f.), *Treponema denticola* (T.d.). The participants were asked to refrain from oral hygiene measures, two hours before sample collection. Subgingival microbial samples were collected from all the four banded molars and pooled together for ortho group (Figure.2). In the non-ortho group, plaque samples were collected from molar area from each quadrant and pooled together. Supragingival plaque was carefully removed and the sampling sites were isolated with sterile cotton rolls and gentle air drying. A sterile paper point was softly inserted into each pocket for 30 seconds until a minimum of resistance is encountered. These paper points were transferred immediately into Eppendorf tubes containing TE buffer and were kept in a freezer at -80°C. Plaque samples were collected at baseline and after baseline, 1st month, 3rd month and 6th month for analysis of red complex bacteria. DNAs were extracted

from the samples and real-time PCR technique was used to estimate and quantify the red complex bacteria level.

Here we used DNA Isolation Kit Protocol and DNA purification was done by means of DNA Purification from blood or body fluids (Spin Protocol - QIAamp DNA mini kit) at department of biotechnology, SRM institute of science and technology (Figure.3). Real time PCR or Quantitative PCR (qPCR) reaction was carried out through QuantStudio(2). Primers (Figure.4) used for PCR analysis are given in table 9.

All the clinical and microbiological data were collected and transferred into an Excel spreadsheet. The data collected were statistically analysed with software program (SPSS 19.0 version). Clinical parameters were expressed in terms of mean and standard deviation, Microbiological parameters were expressed in terms of C_t Value. Mann Whitney U test was used to compare the clinical parameters and microbiological data between GRP 1 and 2 at various time intervals (Inter group). Friedman test was used to compare within a group at various time intervals (Intra Group). In both the above statistical tools the probability value below 0.05 is considered to be statistically significant.

MICROBIOLOGICAL RESULTS

Red complex bacteria which comprises 3 periodontopathogens were detected and quantified using Real time PCR technique. They were *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*. The results were expressed as C_t values. It was inversely proportional to bacterial count.

Note: The threshold cycle (C_t) is the cycle number at which the fluorescent signal of the reaction crosses the threshold. The C_t is used to calculate the initial DNA copy number, because the C_t value is inversely related to the starting amount of target.

P.gingivalis:

When compared between 2 groups: group 1 showed low mean $\pm$ standard deviations of C_t values (which is inversely proportional to bacterial count). But there was no significant increase in bacterial count

at baseline and at the end of 1 month ($P=0.862$, $P=0.862$ respectively).

The bacterial count of group 1 at the end of 3rd month were increased in a highly statistically significant manner ($P=0.013$) and also the same at the end of 6 months ($P=0.040$). C_t value difference between two groups was 1.81 ± 0.54 at T6. (Figure.5)

Tannerella forsythia:

In intergroup comparison of 2 groups, even though group 1 showed less bacterial count at baseline and at the end of 1 month there was a gradual increase over the time period. But the comparative elevation of bacterial levels in group 2 was not statistically significant. There was a highly significant increase of bacterial levels in group 1 at the end of 3rd and 6th month ($P=0.032$, $P=0.026$ respectively). (Figure.6)

Treponema denticola:

When compared between 2 groups: group 1 showed more bacterial counts at baseline, at the end of 1 month and 3 months, but not statistically significant. Likewise there was not much change of C_t values when two groups are matched. At the end of 6 months there was a statistically significant increase of bacterial levels in group 1 ($P=0.021$). (figure.7)

CLINICAL RESULTS

PLAQUE SCORES:

When plaque scores were compared among 2 groups: Group 1 shows low plaque scores than Group 2 at baseline and increased by the end of 1 month but statistically non-significant ($P=0.728$). Plaque scores were significantly increased in Group 1 by the end of 3 months and 6 months with P value of 0.009 and 0.008 respectively. (figure.8)

GINGIVAL SCORES:

When gingival scores were compared among 2 groups: gingival scores were increased at baseline, by the end of 1 month, and 3 months but

no statistical significance was noted with a P value of 0.132, 0.839 and 0.183 respectively. At the end of 6th month high statistical significance was achieved ($P=0.005$) with a gingival score of 0.86 ± 0.03 was observed in group 1 while in group 2 it was 0.70 ± 0.03 .(figure.9)

BLEEDING ON PROBING SCORES (BOP):

When compared between 2 groups: BOP scores were increased in group 1 than group 2 at baseline, at the end of 1 month and 3 months, but not statistically significant with a P value of 0.450 and 0.154 respectively. But slight shift towards significance was observed at the end of 6th month. ($P=0.05$)(figure.10)

POCKET PROBING DEPTH (PPD):

When PPD were compared among 2 groups: PPD scores were increased in group 1 with a statistically high significant value at the end of 1 month, 3 months and 6 months ($P=0.015$, 0.001, 0.001 respectively). At the end of 6th month there was a mean difference of 1.72 ± 0.02 mm of PPD & 2.16 ± 0.01 mm CAL was observed in ortho patients compared to untreated. (figure.11)

CLINICAL ATTACHMENT LOSS (CAL):

Inter-group comparison showed statistically very significance level ($P=0.001$) In group 1 there was a mean increase of CAL by 3.36 ± 0.01 mm when calculated from baseline to T6 whereas in group 2 was only 0.12 ± 0.02 mm. (figure.12)

Discussion

It has been reported that orthodontic bands can compromise the periodontal health of the surrounding tissues. However, several studies reported only clinical changes without microbiological evaluation around orthodontic bands. Regardless of the quality of plaque control, most subjects undergoing fixed orthodontic treatment develop generalized gingi-

vitis within a short time. The qualitative change in the microbiota, which involves the growth of periodontopathogenic bacteria, could be associated with the gingival inflammation around the orthodontic bands.

The clinical results of this study specified that the plaque scores, gingival scores, bleeding on probing, pocket probing depth and CAL were increased significantly ($P=0.0001$) from baseline to 1- month, 3-months and 6-months in both groups over the recommended time period. Our study is in agreement with several previous studies, (**Huser et al 1990**(3), **Alexander et al 1990**(4), **Boyd 1989**(5) molar bands have been shown to cause statistically significant increase of clinical parameters compared with untreated patients. **Suhad M et al 2008**, (6) also found that during and at the end of the treatment, PI, GI, PPD and CAL were pointedly greater for banded molars than for analogous bonded molars. During treatment there was an increase of 1.5 mm PPD than pre-treatment time and it became around 4 mm by end of the treatment. In our study PPD increased to 5.01 ± 0.20 mm from a baseline value of 2.42 ± 0.15 mm.

In accordance with our study **Naga sri et al, 2010** (7) found that following molar banding, there was substantial rise in plaque scores, gingival scores and pocket probing depths in ortho groups than in controls. According to our results PI score almost doubled in ortho group when compared to baseline by a mean change of 0.46 ± 0.03 . Similarly **Naser et al, 2014**(8) also determined a significant increase of PI, GI and PPD in patients undergoing orthodontic therapy. **Saud A. Al-Anezi et al, 2014**(9) also observed a significant increase in all clinical parameters in patients undergoing fixed orthodontic therapy.

Bilimoria, 1963(10) found a noteworthy relationship between malocclusion and periodontal disease. Likewise in our study also there was a significant elevation in all clinical parameters with a P value of 0.001 over the recommended time period in malocclusion patients without treatment. There was a significant increase of PPD and CAL by 1.74 ± 0.05 mm over 6 months. Similarly **Buckley et al, 1972** (11) also concluded that malocclusion would be more likely to be linked with increased periodontal disease.

Our results could hypothesize that more plaque accumulation and rise in clinical parameters in orthodontic patients with molar bands may be due to difficulty in oral hygiene maintenance. Fixed orthodontic appliances like molar bands and brackets are a hindrance to adequate oral hygiene practice and thereby results in retention of plaque, and ultimately leads to periodontal breakdown. Difficulty in oral hygiene maintenance can be assumed as a major risk factor which should be taken into consideration while undergoing fixed orthodontic therapy.

Skillin, 1940 (12) and Reitan, 1957(13) confirmed that most of the periodontal tissue alterations that occur during orthodontic treatment are reversible. Our results differ from the observations by **Diamanti-Kipiotti et al, 1987 (14)** who, in a longitudinal study, found no significant variations in plaque or gingival scores after initiation of orthodontic treatment. This discrepancy between the studies may be related to differences in age or host-resistance factors in the patient populations. Also, the time necessary for the development of gingival inflammation, when oral hygiene is abolished, varies from one person to another and depends on the rate of plaque formation. Similar study done by **Shrestha S et al, 2016 (15)** concluded that there is no significant change in clinical parameters in molar banded areas when oral hygiene is controlled. **Krisztina Martha et al, 2016(16)** found that harmful outcomes are reversible after debonding.

Analyzing the initial biofilm composition (T0) our results showed that the red complex bacteria were present in the subgingival plaque of all patients (100% prevalence). The load of red complex bacteria was significantly increased in both orthodontically treated and untreated patients but comparatively more significant elevation was observed in ortho treated patients with molar bands when compared to untreated.

Previous studies had also proved similar results which is in accordance with our study. **Petti et al, 1997(17)** presented significantly increased counts of *P. gingivalis* when the effect of orthodontic therapy with fixed and removable appliances on oral microflora over a six-month period was assessed. **Bloom and Brown, 1964 (18)** observed a generalized

increase in all bacterial counts after band placement, which is in accordance with this study. **Flores De Jacoby & Muller, 1982(19)** also observed this “shift” in microorganisms from aerobes to anaerobes. Consistent with our results, **Anhoury P et al, 2002 (20)** reported significantly higher ($P < 0.05$) mean counts of *Treponema denticola* was 0.14×10^5 from molar bands and brackets at the end of orthodontic treatment.

In agreement with our results **Amit Sidana et al, 2017(21)** concluded that noteworthy differences were illustrated between the red complex bacterial level in pre-treatment group and active treatment group. **Pooja Desai et al, 2018 (22)** found that the combination of the occurrence of red complex bacteria in subgingival plaque samples was considerably associated with clinical parameters. **M Franch et al, 2019(23)** established a direct relation between the placement of fixed appliance and the increase of plaque index and gingival index. Also, the growth of periodontopathogens in subgingival plaque is promoted. Similarity in the way of microbiological analysis can be found since in many recent studies it is done by PCR technique.

Conversely in a study proposed by **David Jolley et al, 2016 (24)** greater amount of periodontal pathogens were detected in the orthodontic sample. Interestingly, even higher levels were noticed in the non-orthodontic sample, when comparing the two. This suggests that orthodontic patients could benefit from not only routine dental and periodontal therapy, but also from better education and awareness regarding the chance of increased risk for the development of colon cancer among some patients. **Shuang Pan et al, 2017(25)** also has the same opinion. But according to him and **Hongyan Liu et al, 2010(26)** the microorganisms persisted at high levels for 6 months after appliance removal. This might imply a potential risk to periodontal condition in certain patients. So these literatures explained the importance of maintaining our gingival health and difficulty of recovery once the disease is gone advanced.

All the above clinical trials including our study speculated the relevance of routine clinical examination and microbiological evaluation during active orthodontic therapy. Improving patient compliance who have been aware of his/her correct periodontal health

profile is a major advantage of this assessment. Proper oral hygiene practices and frequent visit to a periodontist can help us stay away from permanent disease risks.

CONCLUSION

Results showed that proper oral hygiene measures and special attention are necessary for both ortho patients and malocclusion patients. Periodontal health of patients undergoing fixed orthodontic therapy as well as untreated malocclusion patients should be monitored frequently to assess the subsequent damage to the periodontium. Thus, patient motivation and oral hygiene awareness are essential elements to preserve the periodontal health. The results emphasize the need for oral prophylaxis and maintenance of oral hygiene by having frequent examination of periodontal condition by a periodontist. Necessary steps to improve oral hygiene have to be taken and control the potential causes if any severe problems are observed. This helps to prevent possible progress of the periodontal destruction.

Main points

- Proper oral hygiene measures and special attention are necessary for both ortho patients and malocclusion patients.
- Study reveals the importance of oral hygiene maintenance and frequent monitoring of periodontal health especially in patients with orthodontic appliances and malaligned teeth.
- **Orthodontic bands and brackets act as a niche for plaque accumulation and elevated growth of periodontopathogens.**

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FIGURE LEGENDS

1. clinical Assessment
2. Collection of subgingival plaque samples
3. Spin Protocol - QIAmp DNA mini kit
4. Primers
5. Inter Group Comparison of P.gingivalis bacteria level at various intervals
6. Inter Group Comparison of T.forsythia bacteria level at various intervals
7. Inter Group Comparison of T.denticola bacteria level at various intervals
8. Inter Group Comparison of Plaque scores at various intervals
9. Inter Group Comparison of Gingival scores at various intervals
10. Inter Group Comparison of BOP scores at various intervals
11. Inter Group Comparison of PPD scores at various intervals
12. Inter Group Comparison of CALscores at various intervals

Note: X-axis and Y-axis indicate time intervals and Ct values respectively in case of microbial analysis. Clinical parameter analysis

graphs depicted time interval in X- axis and scores at the Y-axis



Fig 1: clinical assessment



Fig 2: subgingival sample collection



Fig 3: Spin Protocol - QIAamp DNA mini kit



fig 4: primers

Note: X-axis and Y-axis indicate time intervals and Ct values respectively in case of microbial analysis. Clinical parameter analysis

graphs depicted time interval in X- axis and scores at the Y-axis

Fig 5: Inter Group Comparison of *P. gingivalis* bacteria level at various intervals

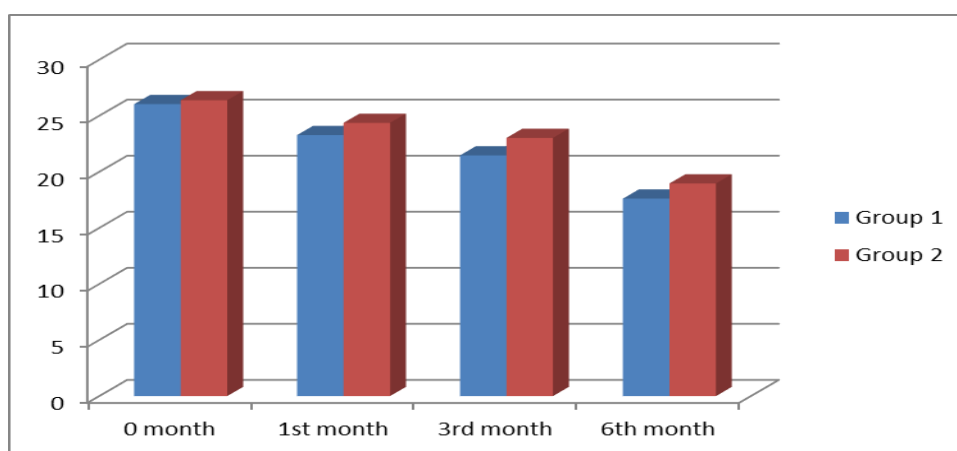


Fig 6 : Inter Group Comparison of *T. forsythia* bacteria level at various intervals

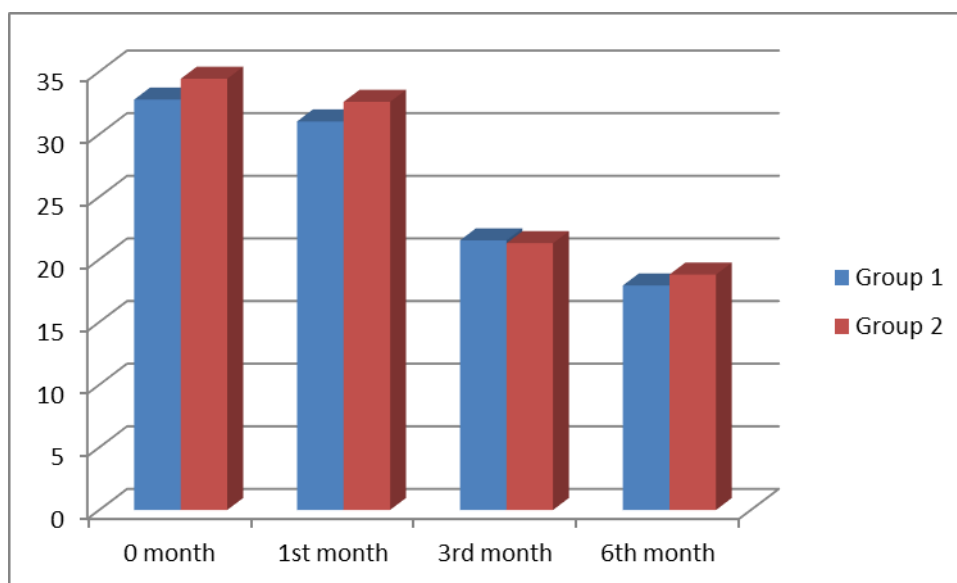


Fig 7: Inter Group Comparison of *T. denticola* bacteria level at various intervals

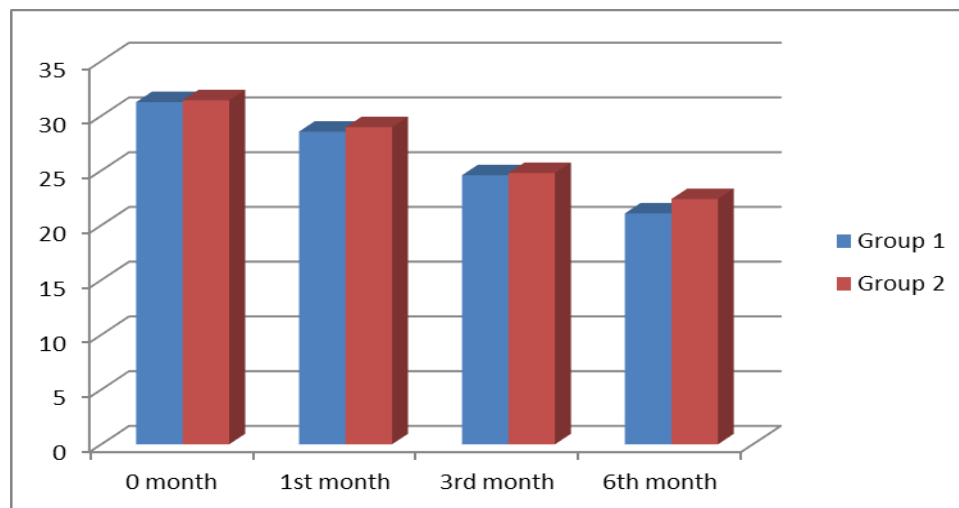


Fig 8: Inter Group Comparison of Plaque scores at various intervals

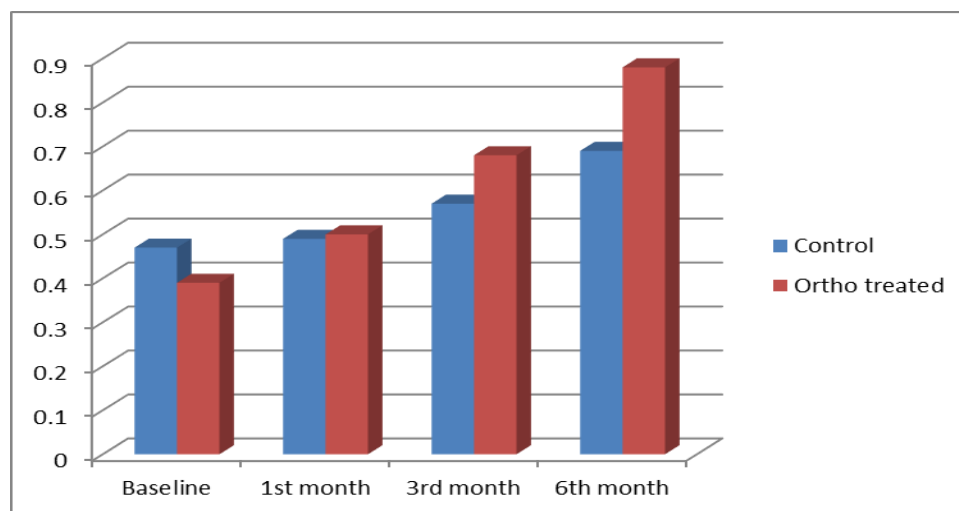


Fig 9: Inter Group Comparison of Gingival scores at various intervals

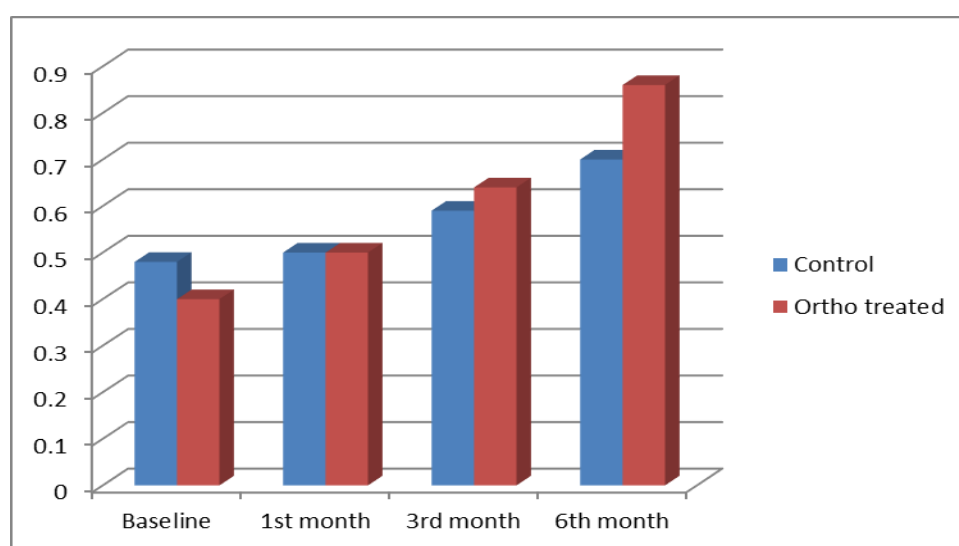


Fig 10: Inter Group Comparison of BOP scores at various intervals

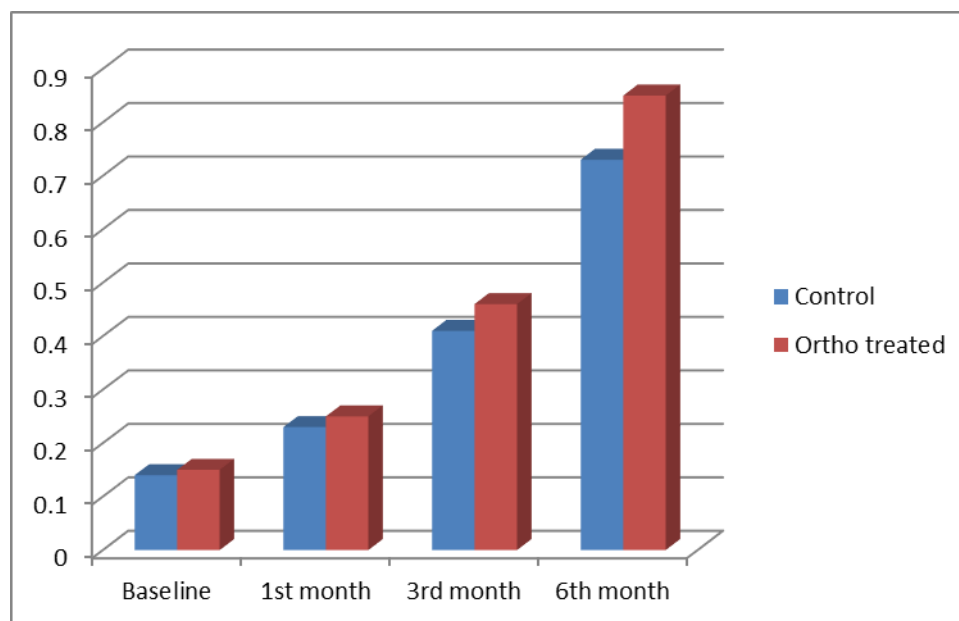


Fig 11: Inter Group Comparison of PPD scores at various intervals

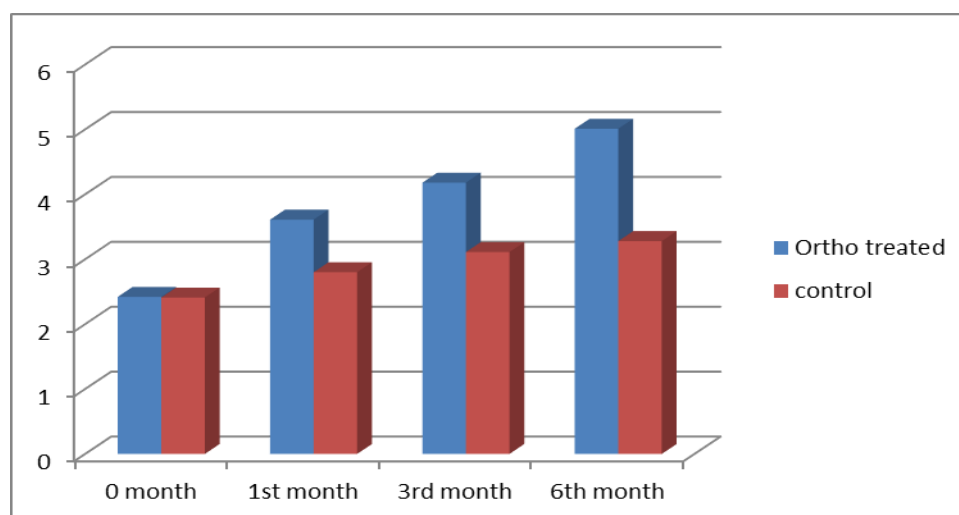


Fig 12: Inter Group Comparison of CAL scores at various intervals

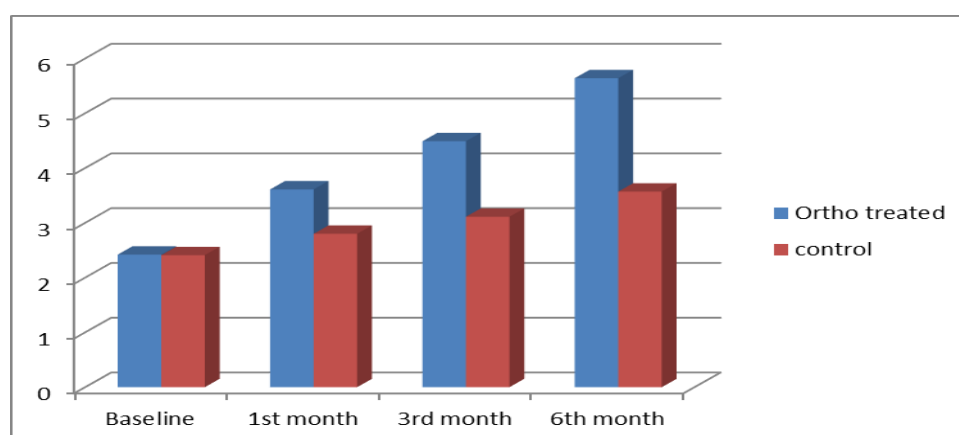


Table no 1 : Inter Group Comparison of P.gingivalis bacteria level at various intervals

Time	Group 1 (mean±SD)	Group 2 (mean±SD)	Mann Whitney U test	P value
0 month	26.04 ± 0.74	26.39 ± 0.45	69.000	0.862
1 st month	23.28 ± 0.46	24.37 ± 0.37	41.000	0.073
3 rd month	21.47 ± 0.36	23.03 ± 0.42	29.000	0.013 (S)
6 th month	17.17 ± 0.39	18.96 ± 0.62	38.000	0.040 (S)

Table 2: Inter Group Comparison of T.forsythia bacteria level at various intervals

Time	Group 1 (mean±SD)	Group 2 (mean±SD)	Mann Whitney U test	P value
0 month	32.77 ± 0.54	34.43 ± 0.66	39.000	0.057
1 st month	31.02 ± 0.62	32.59 ± 0.73	40.000	0.065
3 rd month	21.12± 0.28	22.98 ± 0.23	58.000	0.032 (S)
6 th month	17.12 ± 0.39	18.98 ± 0.49	47.000	0.026 (S)

Table 3 : Inter Group Comparison of T.denticola bacteria level at various intervals

Time	Group1 (mean±SD)	Group2 (mean±SD)	Mann Whitney U test	P value
0 month	31.26 ± 0.26	31.42 ± 0.29	68.000	0.817
1 st month	28.56 ± 0.36	28.96 ± 0.48	59.000	0.453
3 rd month	24.21 ± 0.31	24.97± 0.29	71.000	0.064
6 th month	21.09 ± 0.77	22.98 ± 0.75	45.000	0.021 (S)

Table 4 : Inter Group Comparison of Plaque scores at various intervals

Time	Group 1 (mean±SD)	Group 2 (mean±SD)	Mann Whitney U test	P value
Baseline	0.47 ±0.03	0.39 ± 0.04	45.000	0.118
1 st month	0.49 ± 0.03	0.50 ± 0.03	66.000	0.728
3 rd month	0.57 ± 0.02	0.68 ± 0.03	27.000	0.009 (S)
6 th month	0.69 ± 0.03	0.88 ± 0.05	26.500	0.008 (S)

Table 5: Inter Group Comparison of Gingival scores at various intervals

Time	Group 1 (mean±SD)	Group 2 (mean±SD)	Mann Whitney U test	P value
Baseline	0.48 ± 0.02	0.40 ± 0.04	46.000	0.132
1 st month	0.50 ± 0.02	0.50 ± 0.03	68.500	0.839
3 rd month	0.59 ± 0.02	0.64 ± 0.03	49.000	0.183
6 th month	0.70 ± 0.03	0.86 ± 0.03	23.000	0.005 (S)

Table 6: Inter Group Comparison of BOP scores at various intervals

Time	Group 1 (mean±SD)	Group 2 (mean±SD)	Mann Whitney U test	P value
Baseline	0.14 ± 0.01	0.15 ± 0.01	57.000	0.386
1 st month	0.23 ± 0.01	0.25 ± 0.01	59.000	0.450
3 rd month	0.41 ± 0.03	0.46 ± 0.02	47.500	0.154
6 th month	0.73 ± 0.04	0.85 ± 0.02	38.500	0.053

Table 7: Inter Group Comparison of PPD scores at various intervals

Time	Group 1 (mean±SD)	Group 2 (mean±SD)	Mann Whitney U test	P value
0 month	2.42 ± 0.15	2.41 ± 0.14	63.500	0.623
1 st month	3.61 ± 0.20	2.80 ± 0.17	30.000	0.015
3 rd month	4.18 ± 0.19	3.11 ± 0.18	16.000	0.001 (S)
6 th month	5.01 ± 0.20	3.28 ± 0.18	5.000	0.0001 (S)

Table 8: Inter Group Comparison of CAL scores at various intervals

Time	Group 1 (mean±SD)	Group 2 (mean±SD)	Mann Whitney U test	P value
Baseline	2.42 ± 0.15	2.41 ± 0.14	63.500	0.623
1 st month	3.61 ± 0.20	2.80 ± 0.17	30.000	0.015 (S)
3 rd month	4.49 ± 0.25	3.11 ± 0.18	13.000	0.001 (S)
6 th month	5.64 ± 0.16	3.57 ± 0.32	12.000	0.001 (S)

Target organism	Sequence (5' 3')
T.forsythia Forward	GCGTATGTAACCTGCCCCGA
T.forsythia Reverse	CCGTTACCTCACCAACTACCTAATG
T.denticola Forward	TAATACCGAATGTGCTCATTACAT
T.denticola Reverse	TCAAAGAAGCATTCCTCTTCTTCTTA
P.gingivalis Forward	CTTGACTTCAGTGGCGGCAG
P.gingivalis Reverse	AGGGAAGACGGTTTTACCA