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# Evaluating and Comparing Periodontal and Microbial Parameters in Orthodontic Patients with Elastomeric Modules and Self Ligating Brackets- A PCR Study

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## Abstract

**Introduction and Objectives:** In fixed orthodontic appliance system, arch wires are held to brackets with either stainless steel ligature wires or elastomeric modules. Self-ligating brackets do not require an elastic or wire ligature. The method of ligation has been shown to be associated with changes in microbial flora. Hence present study was conducted to evaluate and compare periodontal and microbial parameters in orthodontic patients with elastomeric modules and self ligating brackets.

**Materials and Methods:** Sixteen patients scheduled for fixed orthodontic treatment were included. Group I consisted of 8 patients (mean age 15.2 yrs) treated by conventional stainless steel brackets ligated by the elastomeric modules and Group II consisted of 8 patients (mean age 17.7 yrs) treated by stainless steel self ligating brackets. Clinical parameters; Plaque index (PI), gingival index (GI), and pocket depth (PD) were measured and subgingival samples were taken before placement of orthodontic appliances (T0), and 5 week (T1), 12 weeks (T2), after placement of orthodontic appliances in both the groups. PCR technique was used to evaluate the *Aggregatibacter actinomycetemcomitans* (AA), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), and *Streptococcus mutans* (SM).

**Results:** There was a significant increase in Plaque index, gingival index, and pocket depth during the 12 weeks of appliance placement in both groups. Pocket depth was significantly increased in conventional group when compared with self ligating bracket group. The relative quantity of *A. actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi) were high at 12 weeks orthodontic treatment in conventional group. The difference in bacterial count was not found statistically significant in two groups.

**Conclusions:** The ligation method was found to be a contributing factor for plaque accumulation and deteriorating gingival condition. Brackets with elastomeric modules were found be associated with increased mean values of clinical parameters and anaerobic bacterial count as compared with that of self ligating bracket group.

***Keywords:*** *Self Ligating Brackets; Plaque Index; Microbacteria; PCR Study*

## Introduction

Fixed orthodontic treatment involves the use of controlled forces acting on teeth and associated structures and thereby moving them to desired position. Tooth movement is achieved through alveolar bone remodelling which is primarily mediated through cascade of tissue reactions occurring in periodontal ligament. Therefore healthy gingiva and periodontium are the prime requirements to achieve optimal orthodontic treatment outcome.

The design and surface characteristics of fixed orthodontic attachments and the composite used for bonding increase the plaque retention. Orthodontic appliances severely hamper the efficacy of tooth brushing and the self-clearance by saliva. Therefore combination of orthodontic therapy for a period of 1 - 2 year and poor oral hygiene could cause serious damage to periodontium by accumulation of microbial dental plaque [1]. The resultant inflammatory condition such as gingivitis, also delays the response to forces applied for tooth movement.

In routine fixed orthodontic appliance system, arch wires are held to brackets with either stainless steel ligature wires or elastomeric modules. Self-ligating brackets do not require an elastic or wire ligature. The method of ligation has been shown to be associated with changes in microbial flora [2]. However few studies have compared the effects of arch wire ligation [2-4]. The present study investigated the advantage of self-ligating brackets in terms of the accumulation of plaque because of the absence of ligatures.

In previous studies, although various techniques have been used for the assessment of microbial flora, the microbiologic culture technique was the most widely used [2,3,5-8]. However, the laboratory procedures for this technique can be faulty, time-consuming, and laborious. Recently, to overcome these limitations, polymerase chain reaction (PCR) has been used. PCR is a simple, fast, and accurate method for identification and detection of microorganisms [9]. The present study utilized this technique for microbial evaluation. Therefore, the aim of this study was to evaluate the effects of self-ligating brackets and conventional brackets ligated with elastomeric modules on dental plaque retention and microbial flora using PCR technique.

## Materials and Method

Total sample of sixteen patients (mean age of 16 yrs) who reported for orthodontic treatment were included in the study. The patients were given a written explanation on the background of study, its objectives, their involvement and were asked to give written informed consent. The inclusion criterion for the study were; patients requiring fixed orthodontic treatment, healthy periodontium with pockets ≤ 3 mm, no antibiotics taken within preceding 3 months to start of the study, received no periodontal therapy in last three months, and willing to follow advised plaque control regimen, along with good general health. Patients suffering from systemic disease or taking medication for the same, heavy smokers or consuming tobacco, taken any antibiotic within three months prior to this study, with a previous history of periodontal surgery, and teeth with extensive restorations or crowns were excluded from the study.

Two weeks before the baseline examination, all subjects underwent one session of accurate supragingival and subgingival ultrasonic scaling with proper oral hygiene instructions. The use of a mouthwash was not allowed for entire duration of study. The total sample of sixteen participants was randomly divided in to two equal groups. In one group (n = 8) conventional stainless steel brackets (Ormco Mini 2000) were bonded and the elastomeric modules were used for ligation of arch wire and second group (n = 8) of participants received stainless steel self ligating brackets (RMO FLI Self Ligating Brackets).

The clinical examination consisted of recording the Gingival index [10], Plaque index [11] and probing depth before collecting samples. The probing depth was measured around teeth from right first molar to left first molar in both arches on buccal/labial(mesial, mid distal) and mid palatal/lingual areas using a marked periodontal probe (UNC-15, Hu Friedy, Chicago, Ill). Plaque sample for microbiological evaluation was collected from buccal surfaces of pre-molars and lateral incisors in both arches. The plaque samples were obtained with insertion of sterile paper points subgingivally from buccal/labial surface of selected teeth (Figure 1). This paper point was left for 30 seconds, then carefully removed and transferred to vials containing Tris-EDTA buffer tr. Transport media (Figure 2). Then, the vials were designated with unique participant identification code and dispatched to microbiological lab.

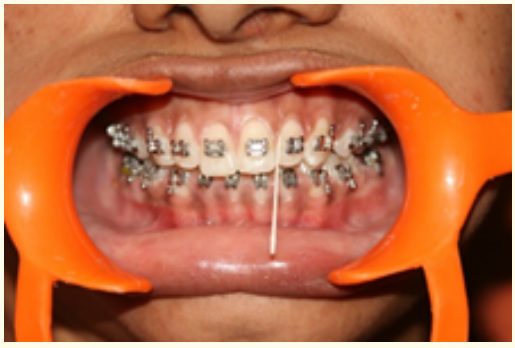

***Figure 1:*** *Collection of plaque samples with sterile paper points subgingivally from buccal/labial surface of teeth.*

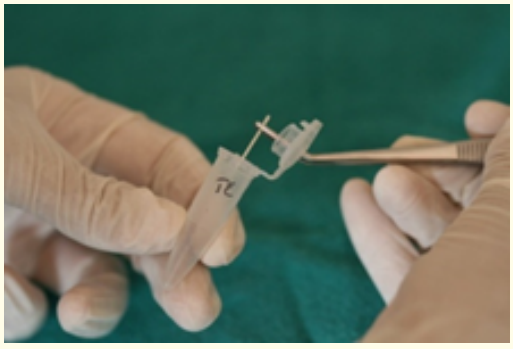

***Figure 2:*** *Tris-EDTA buffer Transport media.*

In microbiological lab, the samples were centrifuged at 5,000 rpm for 5 minutes. The supernatant was discarded and 500 µL fresh Tris EDTA buffer was added. It was then centrifuged for 3 - 4 minutes. Supernatant was discarded and 50 µL lysis buffer I (1M Tris buffer 500 µL, Triton X-100 - 500 µL, 0.5M EDTA 100 µL, Distilled Water- Made to 50 mL) was added, then vortex was done and kept for 5 minutes. Then, 50 µL Lysis buffer II (Tris HCL 50 mM (pH 8.0), KCL- 50 mM, $MgCl_2$-2.5 mM, Tween 20- 0.45%, Nodient P-40- 0.45%) and 10 µL proteinase – K (100 µg/mL) were added. The vortex was done vigorously. It was kept in water bath for 2 hrs and then kept in boiling water bath for 10 minutes. The DNA was stored at -20$^0$C.

The primers applied in PCR were designed by Oligonucleotide Primers: Bioservepvt Ltd (Table 1). PCR reaction was set up in a total volume of 50 µL /aliquot. (QiagenTaq core kit - 3U/µL Taq Polymerase, 2 Coral load buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP.). The cycling conditions used were- Initial denaturation at 95$^0$C for 5 minutes; Annealing at 60°C for 1 minute, and final extension procedure at 72$^0$C for 5 minutes. PCR procedure was repeated for 40 cycles (Thermal cycler: Applied Biosystems, USA). The samples were kept at 4$^0$C following PCR. The electrophoresis and gel documentation procedure (Documentation system: Major Science, USA) were done for identification and quantification of amplified products.

| Bacterias and Primer Sequence | Product Size |
|---|---|
| **1) Porphyromonas gingivalis**<br>Forward primer: AGG CAG CTT GCC ATA CTG CG<br>Reverse primer: ACT GTT AGC AAC TAC CGA TGT | 404 |
| **2) Actinobacillus actinomycetemcomitans**<br>Forward primer: GCT AAT ACC GCG TAG AGT CGG<br>Reverse primer: ATT TCA CAC CTC ACT TAA AGG T | 443 |
| **3) Prevotella intermedia**<br>Forward primer: AAC GGC ATT ATG TGC TTG CAC<br>Reverse primer: CTC AAG TCC GCC AGT TCG CG | 598 |
| **4) Streptococcus mutans**<br>Forward primer-ACTACACTTTCGGGTGGCTTGG<br>Reverse primer-CAGTATAAGCGCCAGTTTCATC | 517 |

***Table 1:*** *PCR primers used in the study.*

### Statistical analysis

Statistical software SPSS version 16.0 was used for statistical analysis. For clinical parameters, intragroup comparisons were performed by applying Paired t test and intergroup comparisons were performed by Student's t test. Nonparametric tests were used for microbial parameters. Statistically significant differences for microbiologic data between groups were determined using the Mann-Whitney U test. The Wilcoxon signed rank test was used to determine the differences in the mean changes within each group. The significance for all statistical tests was predetermined at $P \leq 0.05$.

## Results

Table 2-4 shows inter group and intragroup comparison of Plaque index (PI), gingival index (GI), and pocket depth (PD) in two groups. Plaque index (PI), gingival index (GI), and pocket depth (PD) were low before commencement of treatment. After 12 weeks (T3) of appliance wear, there was a significant increase in PI, GI, PD in both the group. Table 4 shows inter-group comparison indicating significant increase in probing depth in conventional group as compared with self ligating brackets.

| | Group | N | Mean | Std. Deviation | p-value |
|---|---|---|---|---|---|
| Difference between gingival index at base line (T0) | Conventional Group (group-I) | 8 | 0.11 | 0.12 | 0.948 (NS) |
| | Self ligating (Group II) | 8 | 0.11 | 0.10 | |
| Difference between gingival index at 5 weeks (T1) | Conventional Group (group-I) | 8 | 1.04 | 0.38 | 0.536 (NS) |
| | Self ligating (Group II) | 8 | 0.93 | 0.29 | |
| Difference between gingival index at 12 weeks (T2) | Conventional Group (group-1) | 8 | 1.56 | 0.37 | 0.434 (NS) |
| | Self ligating (Group II) | 8 | 1.44 | 0.25 | |

***Table 2:*** *Intergroup comparison of Conventional MBT group (Group-I) and self ligating bracket group (Group-II) of Gingival Index evaluated at different time intervals. (Base line (T0), at 5weeks (T1), at 12 weeks (T2)*

*The level of *p ≤ 0.05 ,**p ≤ 0.01, ***p ≤ 0.001 were significant and p > 0.05 were non significant (N.S)*

| | Group | N | Mean | Std. Deviation | p-value |
|---|---|---|---|---|---|
| Difference between Plaque index at base line (T0) | Conventional Group (group-I) | 8 | 0.41 | 0.24 | 0.953 (NS) |
| | Self ligating (Group II) | 8 | 0.42 | 0.26 | |
| Difference between Plaque index at 5 weeks (T1) | Conventional Group (group-I) | 8 | 1.64 | 0.21 | 0.312 (NS) |
| | Self ligating (Group II) | 8 | 1.47 | 0.40 | |
| Difference between Plaque index at 12 weeks (T2) | Conventional Group (group-1) | 8 | 1.99 | 0.28 | 0.074 (NS) |
| | Self ligating (Group II) | 8 | 1.74 | 0.22 | |

***Table 3:*** *Intergroup comparison of Conventional MBT group(Group-I) and self ligating bracket group (Group-II) of plaque index evaluated at different time intervals. Base line (T0), at 5weeks (T1), at 12 weeks (T2).*

*The level of *p ≤ 0.05 ,**p ≤ 0.01, ***p ≤ 0.001 were significant and p>0.05 were non significant (N.S)*

| | Probing depth | Mean | Std. Deviation | Mean Difference | p-value |
|---|---|---|---|---|---|
| Difference between Probing depth taken at base line (T0) & after 5 weeks (T1) | Probing depth at base line (T0) | 1.846 | 0.415 | 0.546 | 0.001*** |
| | Probing depth after 5weeks (T1) | 2.393 | 0.336 | | |
| Difference between Probing depth taken at base line (T0) & after 12 weeks (T2) | Probing depth at base line (T0) | 1.846 | 0.415 | 1.29 | 0.001*** |
| | Probing depth after 12 weeks (T2) | 3.136 | 0.448 | | |
| Difference between Probing depth taken after 5 weeks (T1) & after 12 weeks (T2) | Probing depth after 5weeks (T1) | 2.393 | 0.336 | 0.743 | 0.005** |
| | Probing depth after 12 weeks (T2) | 3.136 | 0.448 | | |

***Table 4:*** *Intragroup comparison of Probing depth(PD) evaluated at different time intervals in conventional bracket group (Group-I). Base line T0, at 5weeks (T1), at 12 weeks (T2)*

*The level of *p ≤ 0.05 ,**p ≤ 0.01, ***p ≤ 0.001 were significant and p > 0.05 were non significant (N.S)*

Table 5 shows the descriptive statistics and an intragroup and intergroup comparison of the microbiological measurements in both groups. After bonding of the orthodontic brackets, total bacterial count was found increased in both the groups under study but difference was significant only in self ligating group at 12 weeks (T3). Intergroup comparison did not found any significant difference.

Table 5 shows mean bacterial counts of *A. actinomycetemcomitans* (Aa) and *P. gingivalis* (Pg). They were found to be increased in conventional group from base line (T0) to 12 weeks period (T2) as compared with self ligating bracket group. Mean bacterial count of *Prevotella intermedia* was found to be higher in self ligating bracket group as compared to conventional group. Although intergroup and intragroup comparisons did not reveal statistical significant differences. *Streptococcus mutans* counts in both the groups showed significant increase from base line (T0) to 12 weeks period (T2).

| Groups | At base line (T0) | After 5 week (T1) | After 12 week (T2) | Significance between | | |
|---|---|---|---|---|---|---|
| | | | | T0-T1 | T0-T2 | T1-T2 |
| Total Bacterial Count | | | | | | |
| Conventional Group (Group-I) | 2.96 ± 7.79 | 4.29 ± 11.76 | 6.12 ± 15.98 | 1 (NS) | 0.918 (NS) | 0.279 (NS) |
| Self Ligating Bracket Group (Group-II) | 0.051 ± 0.133 | 1.156 ± 3.669 | 0.588 ± 0.997 | 0.114 (NS) | 0.006* | 0.182 (NS) |
| P value | | | | 0. 019* | 0.74 (NS) | 0.73 (NS) |
| *Actinobacillus actinomycetemcomitans* | | | | | | |
| Conventional Group (Group-I) | 0.005 ± 0.01 | 0.002 ± 0.00 | 2.58 ± 10.24 | 0.28 (NS) | 0.345 (NS) | 0.068 (NS) |
| Self Ligating Bracket Group (Group-II) | 0.00 ± 0.00 | 0.25 ± 0.95 | 0.24 ± 0.94 | 0.18 (NS) | 0.11 (NS) | 1 (NS) |
| P value | | | | 0.151 (NS) | 0.502 (NS) | 0.63 (NS) |
| *Porphyromonas gingivalis* | | | | | | |
| Conventional Group (Group-I) | 2.931 ± 7.77 | 4.25 ± 11.74 | 3.31 ± 12.98 | 0.51 (NS) | 0.86 (NS) | 0.18 (NS) |
| Self Ligating Bracket Group (Group-II) | 0.013 ± 0.053 | 0.44 ± 1.17 | 0.003 ± 0.008 | 0.65 (NS) | 0.21 (NS) | 0.59 (NS) |
| P value | | | | 0.056 | 0.463 (NS) | 0.06 (NS) |
| *Prevotella intermedia* | | | | | | |
| Conventional Group (Group-I) | 0.003 ± 0.00 | 0.00 ± 0.00 | 0.028 ± 0.08 | 0.18 (NS) | 0.17 (NS) | 0.06 (NS) |
| Self Ligating Bracket Group (Group-II) | 0.019 ± 0.075 | 0.39 ± 1.52 | 0.046 ± 0.17 | 0.59 (NS) | 0.59 (NS) | 1 (NS) |
| P value | | | | 0.602 (NS) | 0.15 (NS) | 0.422 (NS) |
| *Streptococcus mutans* | | | | | | |
| Conventional Group (Group-I) | 0.03 ± 0.06 | 0.05 ± 0.09 | 0.20 ± 0.28 | 0.463 (NS) | 0.011** | 0.033* |
| Self Ligating Bracket Group (Group-II) | 0.02 ± 0.05 | 0.03 ± 0.05 | 0.30 ± 0.43 | 0.484 (NS) | 0.01** | 0.013** |
| P value | | | | 0.409 (NS) | 0.783 (NS) | 0.528 (NS) |

***Table 5:*** *Comparison of Mean Bacterial Counts of Groups at three evaluation times (T0, T1, and T2)*

*The level of *p ≤ 0.05 ,**p ≤ 0.01, ***p ≤ 0.001 were significant and p > 0.05 were non-significant (N.S)*

*#All mean and SD values should be multiplied by $10^5$ to provide actual bacterial count.*

However, intergroup comparison between three time intervals; between base line (T0), after 5 weeks (T1), and after 12 weeks (T2), did not show any significant difference.

## Discussion

The present study evaluated and compared the clinical and microbiological parameters in conventional bracket system ligated with elastomeric modules and self ligating bracket system. Eight patients were included in each group. The clinical and microbial parameters were measured three times during this study; at base line (before bonding, T0), after 5 weeks (T1), and after 12 weeks (T2). The clinical parameters measured were plaque index, gingival index, and pocket depth. For the microbial evaluation, *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Streptococcus mutans* counts were identified and quantified through PCR technique.

During the study, fixed orthodontic treatment resulted in dental plaque accumulation and gingival inflammation with a significant increase in plaque index, gingival index, and pocket depth in a short time after commencement of treatment as compared to base line. However, comparison between two groups under study suggested insignificant difference in gingival and plaque index but significant difference in probing depth, with conventional group showing higher values.

Consistent with present study results, another study suggested a higher retention of plaque accumulation on conventional brackets ligated with elastomeric ligature than on self ligating brackets [12]. However two other studies have reported that there is no difference in plaque aggregation between self ligating brackets and conventional brackets [13,14].

The observed results in present study emphasized the fact that plaque retention was found more around elastomeric modules in conventional brackets than self ligating brackets. The self ligating bracket system completely lacks any additional ligating mechanism, thereby lessens the chances of food entrapment and plaque retention around the brackets. Lower plaque scores in self ligating bracket system can also be explained as this system allowed hygiene measures (tooth brush) to reach in close vicinity to the brackets around its four surfaces and hence better plaque controlling efficiency in this system. It can be speculated that probably the elastomeric modules around conventional bracket group hampered efficient hygiene maintenance around brackets, greater quantity of plaque accumulation causing gingival irritation, hyperplasia, redness, increased bleeding on probing. The association of bacteria within mixed biofilms is not random, rather there are specific associations among bacterial species. Socransky., *et al.* [15,16] recognized closely associated groups of bacterial species and categorise them according to their colonization capacities and virulence. These groups were named after different colors:- Yellow complex (*Actinomyces*, genus *Streptococcus*), Purple complex (*V. parvula*, *Actinomyces odontolyticus*) are early colonizers on the tooth surface. Orange complex (*Fusobacterium nucleatum*, *Prevotella intermedia*, *Prevotella nigrescens*, *Eubacterium nodatum*, *Campylobacter* species), Red complex (*Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*), Green complex (*Capnocytophaga* species, *A. actinomycetemcomitans*, *E. corrodens*) appear in later stages of gingivitis and periodontitis. The orange complex was found closely associated with red complex and showed the strongest relationship with the clinical parameters which are considered most meaningful in periodontal diagnosis such as bleeding on probing and increased pocket depth. Bacteria from different complexes (orange, red, green) were chosen for evaluation. Polymerase chain reaction (PCR) technique was specifically employed because of its higher sensitivity and specificity compared with conventional methods. Also tests that use the RNA/DNA have the advantage of not requiring viable bacteria [17].

An intergroup comparison of two groups showed no significant difference in mean bacterial count at 5 week and 12 week periods. However, it was seen that at both the time periods the mean values of total bacteria's were higher in conventional (Group-I) than self ligating group (Group-II). Anaerobic bacteria such as *Actinobacillus actinomycetemcomitans* (Aa) counts, *Prevotella intermedia* (PI), *Porphyromonas gingivalis* (Pg) were not increased significantly after bonding of fixed appliances in both the groups. The gram positive bacteria, *Streptococcus mutans*, showed significant increase in both the groups. However, intergroup comparison between three time intervals did not show any significant difference in counts of *Streptococcus mutans*. Increase in the percentage of streptococci was found more in self ligating group as compared to increase in potentially pathogenic anaerobic gram negative bacteria which are frequently associated with inflammatory diseases of periodontium. There are studies which identified organisms antagonistic to periodontopathogens [18,19]. With regard to the red complex, *Staphylococcus aureus* and *Streptococcus mutans* isolates inhibited the growth of *T. denticola* and *P. gingivalis*. *Staphylococcus aureus* strains produced a bacteriocin-like inhibitory substance. In the present study increased level of *P. gingivalis* in conventional group could have caused the inhibitory growth of *S. mutans.*

The patients in present study had important periodontopathic bacteria but no signs of periodontal disease. The mere presence of a specific microorganism does not mean that the patient has periodontal disease, but it depends on a complex bacteria-host interaction that modulates the host's response leading to inflammation and further loss of attachment [20-22]. However, if a slight inflammation is not controlled and plaque accumulation continues, this could have a detrimental impact on patient's periodontium.

In a split-mouth study, Pellegrini., *et al.* [14] demonstrated higher total bacteria and oral *S. mutans* colonization in the conventional brackets group at both 1 and 5 weeks after bonding. Another study found no difference in the levels of *S. mutans* between the self-ligating brackets and conventional brackets ligated with stainless steel ligatures evaluated through PCR method [23]. Microbiological analysis by Pejda., *et al.* [13] found a 23.8 times greater chance for the presence of *Actinobacillus actinomycetemcomitans* (Aa) in subgingival plaque

of patients with conventional brackets ligated with stainless steel than in patients with self ligating brackets. Contradictory results found in these mentioned studies could be attributed to differences in study design, material and methods, studied microbes, and statistical analysis.

Role of interdisciplinary approach has been cited in literature for maintenance of periodontal health in orthodontic patients [24]. Clinical findings along with microbiological evaluation pointed to the role that ligation method affected the periodontal health. The conventional brackets with elastomeric modules were found to be associated with increased counts of anaerobic bacteria (*Actinobacillus actinomycetemcomitans, P. gingivalis*). Anaerobic bacteria were considered important pathogens in various periodontal diseases. Although, difference between conventional and self ligating bracket group for mean total bacterial count were not found statistically significant. *S. mutans* was found increased in self ligating group, which is considered more important in development of cariogenic plaque. The observed results pointed to the role of elastomeric modules in development of increased quantity of plaque which harboured more number of pathological bacteria as compared to self ligating bracket system. Therefore, in orthodontic patients in whom, the gingival/periodontal condition is poor even before start of treatment; it is advised to avoid use of elastomeric modules. The self ligating brackets, were also found to induce changes in periodontal microflora but it was of less magnitude as compared to conventional group. It is therefore advised that self ligating brackets should be considered in conditions of compromised periodontal status, or in conditions where poor patient cooperation regarding oral hygiene maintenance is suspected.

The observed results concluded that pathological changes in periodontium were induced by fixed orthodontic appliances in all patients but it was of moderate degree. Therefore periodontal conditions in patients undergoing orthodontic treatment should be monitored carefully. Thus, patient motivation and oral hygiene education are essential elements to a successful orthodontic outcome.

## Conclusions

Following conclusions were drawn from study:

1. The ligation method was found to be a contributing factor for plaque accumulation and deteriorating gingival condition more in conventional brackets with elastomeric modules than self ligating brackets.

2. Clinical parameters; mean gingival index, plaque index and probing depth were found to be increased in conventional bracket group as compared to self ligating bracket group at 5 and 12 weeks period.

3. Mean of total bacterial count, *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* were found higher in conventional group as compared with self ligating group but it was not found statistically significant in two group.

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