Effect of Intraoral Appliance on Oral Health Status and Streptococcus mutans Count: A Longitudinal Study

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Abstract

Objective Appliances used in preventive and interceptive orthodontics, which are often provided to correct or intercept a specific malocclusion or habit, may be detrimental to a child’s oral health. The objective of this study was to evaluate the effect of intra oral appliance wear on the plaque index, gingival index, bleeding on probing, and on levels of salivary Streptococcus mutans.

Materials and Methods A total of 60 children requiring any preventive or interceptive orthodontic treatment (appliance therapy) were randomly divided into two groups, as fixed orthodontic appliance group \(n=30\) and removable appliance group \(n=30\). Preoperative and post operative assessments of patients were done before and after wearing appliances. Assessment was done by determining oral hygiene status and microbiological evaluation of saliva for \(S.\) mutans count. \(S.\) mutans colonies were counted from saliva cultured on mitis-salivarius-bacitracin (MSB) agar.

Statistical Analysis Data that are continuous in nature were summarized as standard deviation and mean. For “within group” comparisons, paired t-tests were used to analyze the continuous data. For “between group” comparisons, unpaired t-tests and Fisher’s exact test were used to analyze the continuous data.

Results Appliance treatment increases plaque and gingival indices. Plaque and gingival index changes 1 month after deployment of both fixed and removable appliances were essentially the same and statistically non-significant with \(p\)-values of 0.412 and 0.965. Bleeding on probing after insertion of both appliances was not statistically significant (\(p=0.423\)). \(S.\) mutans numbers increased following removable and fixed orthodontics. Fixed appliances had more \(S.\) mutans than removable appliances (\(p=0.014\)).

Conclusion The inherent levels of these microorganisms are related with plaque accumulation due to fixed or removable appliances. Increase in the level of plaque and...
microorganisms after fixed and removable appliance therapy can hamper optimum oral health and thus is a concern for pedodontists. During orthodontic therapy, cariogenic pathogens should be reviewed, and to prevent cariogenic pathogens from increasing and to maintain oral hygiene during orthodontic treatment, proper recall visits, patient and parent education, and regular fluoride application are essential.

Introduction

The field of pediatric dentistry is constantly expanding to encompass primary, therapeutic, preventive, and intervention-of dental treatment for children. Prevention and early intervention have gained popularity in recent decades. Interceptive orthodontics refers to “measures undertaken to intercept a malocclusion that has already developed or is developing, and the goal is to restore a normal function,” while preventive orthodontics is the procedure done to preserve the integrity of occlusion that appears normal at a particular time. Appliances used in preventative and interceptive orthodontics, which are often provided to correct or intercept a specific malocclusion or habit, may be detrimental to a child’s oral health. Orthodontic appliances cause plaque formation, bacterial colonization, white spot lesions, enamel loss, and periodontal tissue damage leading to demineralization of enamel. Increase in plaque formation from intraoral appliances shifts aerobic to anaerobic microbiota, affecting hard and soft tissues. The oral cavity is a very rich ecosystem with plenty of microorganisms. Yet, when this environment is compromised, microbiological equilibrium alters, causing “ecological stress” and periodontopathic and cariogenic bacteria development.

Streptococcus mutans is a part of the normal oral flora known to cause decalcification in 50 to 80% of orthodontic patients because of accumulation of cariogenic plaque around the brackets and bands converting decalcified lesions into carious lesions. The derogatory effect of long-term wear of orthodontic appliances is not limited to the tooth surface only. Gingival and periodontal tissues are also affected by these appliances. Most of the patients develop generalized gingivitis after fixed orthodontic appliances regardless of whether the patient has banded or bonded attachments. Probing depth increases after orthodontic treatment because of the increased gingival enlargement after therapy. It has been observed that soon after placement of appliances adverse changes in oral health occur, and these can be evaluated by increased plaque, probing depths, and gingival bleeding.

Many research assessed the impact of fixed orthodontic equipment on oral flora and periodontal health, in permanent teeth or in the older population. Yet relatively few research are done to determine how detachable items affect plaque retention and microbial flora. In addition, conflicting opinions are seen in the studies that have been done over the years. Therefore, the present study has been undertaken to determine the changes in the oral health status and S. mutans counts after insertion of orthodontic appliances in children. The aims and objectives of this study are (1) to evaluate the effect of intraoral appliance wear on the oral health status, that is, (a) plaque index, (b) gingival index, and (c) bleeding on probing and (2) to evaluate the effect of appliance wear on the levels of salivary S. mutans.

Material and Method

This randomized clinical trial was conducted in the department of pediatric and preventive dentistry. A total of 60 children who require any preventive or interceptive orthodontic treatment (appliance therapy) fulfilling the inclusion criteria were randomly selected for the study. An approval was obtained from the ethical committee prior to the onset of the study. Verbal consent from children and signed consent forms from the guardian were obtained after the nature of the study and the possible risks were fully explained. Sample size was calculated using the formula

\[ n = Z^2 \left( \frac{1-p}{2} \right) / l^2, \]

where \( Z = \) standard normal variate at 95% confidence interval (CI), \( p = \) relative proportion, \( q = \) no proportion zone, and \( l = \) allowable error of precision at 95% CI.

Inclusion Criteria

- Patient wearing fixed or removable orthodontic appliances.
- Healthy child with no known systemic disease.
- Cooperative child.
- Preoperative satisfactory oral hygiene.
- Absence of any active carious lesions.

Exclusion criteria

- Patients with known systemic illness.
- Poor oral hygiene.
- Presence of any active carious lesions.
- Patients on antibiotic therapy.
- Patient using mouthwash.

Fig. 1 Group 1: fixed appliances.
In all, 60 children in the age group of 6 to 14 years who require any preventive or interceptive orthodontic treatment (appliance therapy) fulfilling the inclusion criteria were randomly selected for the study. Subjects selected for the study were divided randomly by the sequential number opaque method (SNOE) into two groups. Group 1 (Fig. 1) consisted of 30 subjects requiring fixed orthodontic appliances and group 2 (Fig. 2) consisted of 30 subjects requiring removable orthodontic appliances.

The medium was prepared under the guidance of the head of the department in the department of microbiology. The mitis-salivarius-bacitracin agar was used for the isolation of \textit{S. mutans}. Standard oral hygiene instructions were provided at the beginning of the treatment using a model, with specific orthodontic appliances. Preoperative and postoperative assessments of patients were done prior to initiation of appliance wear and after 4 weeks of wearing appliances, respectively. The assessment was done by oral hygiene status and microbiological evaluation of saliva for \textit{S. mutans} count.

**Oral hygiene status:** This was assessed by the dental plaque index, gingival index, and bleeding on probing.

**Dental Plaque and Gingival Indices**

Plaque and gingival indices of each subject were measured on mesial, distal, buccal, and lingual/palatal surfaces of each teeth using a sterile periodontal probe according to Silness and Loe\textsuperscript{14} at baseline and after 4 weeks of insertion of the intraoral appliance (Fig. 3).

**Bleeding on Probing**

Bleeding on probing in each subject was measured on mesial, distal, buccal, and lingual/palatal surfaces of each teeth using a sterile periodontal probe at baseline and after 4 weeks of insertion of the intraoral appliance.

**Saliva Collection**

A 2-mL unstimulated whole saliva sample of each individual was collected in sterilized tubes. The subjects were asked to rinse their mouth with deionized water for a minute prior to sampling. The pooled saliva was pipetted and collected in a sterile collection tube. As culturing was performed within 30 minutes of collection of samples, no transport medium was used. The microbiological tests were then performed on each sample (Fig. 4).

![Fig. 2 Group 2: removable appliances.](image1)

![Fig. 3 Clinical recording of plaque score.](image2)

![Fig. 4 Method of collection of saliva.](image3)

![Fig. 5 Streptococcus mutans after 30 days in the fixed appliance group.](image4)
Stage 1: The baseline unstimulated saliva was collected from the subjects and inoculated onto MSB agar (►Figs. 5 and 6). The colony counts were obtained by a clinical microbiologist who was blinded to the subject allocation. The existing (baseline) plaque scores, gingival index, and bleeding on probing of all subjects were recorded. Plaque scores were recorded by applying erythrosine disclosing solution (AlphaPlac) with cotton buds. Following this, each subject received a through oral prophylaxis using ultrasonic scaler to achieve a zero-plaque score.

Stage 2: The plaque scores, gingival index, bleeding on probing, and the salivary *S. mutans* colony-forming units (CFU) were again evaluated after a period of 4 weeks (►Figs. 7 and 8).

**Microbiological Method**

The MSB agar was used as a medium for culture of salivary *S. mutans*. From each of the dilutions a 1-µL volume was pipette onto the agar plates. With the help of sterile spreaders, the saliva was evenly spread onto the agar surface. Under 5 to 10% CO₂, the plates were incubated anaerobically for 48 hours at 37°C. The same investigator processed and examined the plate to avoid any kind of bias. *S. mutans* colonies were observed as spherical or round, convex, raised, light blue in color, ranging from a pinpoint to pinhead size in both the groups at baseline and at 4 weeks after wearing of appliances. Identification of *S. mutans* was confirmed by the catalase test and Gram staining of the smear and microscopy.

**Gram Staining**

Each smear of colony was heat fixed and stained for 1 minute with crystal violet. Gram’s iodine was used for 1 minute over the slide followed by washing with water. Acetone was used for 10 to 30 seconds for decolorization of the smear. Again, the smear was counterstained with a dye safranin for 30 seconds after washing with water. Then a smear slide was observed in the microscope. The presence of *S. mutans* after Gram staining was evaluated by purple staining and the presence of ovoid or spherical cells that were arranged in chains or pairs.

**Catalase Test**

Platinum loops from the agar plate were used for picking up the colony. The colony was placed on a clean glass slide over a drop of H₂O₂. No oxygen bubbles were observed, indicating the presence of *S. mutans*.

**Statistical Analysis**

Data that are continuous in nature were summarized as standard deviation and mean. For “within group” comparisons, paired *t*-tests were used to analyze the continuous data. For “between group” comparisons, unpaired *t*-tests and Fisher’s exact test were used to analyze the continuous data. A *p*-value of less than 0.05 was considered significant.
Results

A total number of 60 subjects, 30 subjects in each group, participated in the study. Of this, one subject from each group discontinued the intervention.

The research groups are shown in Table 1 according with the value of bleeding during probing that was obtained on day 1. In group 1 (fixed appliances), bleeding on probing was absent in 25 subjects, which comprised 86.21% of the total subjects, while it was present in 4 subjects, which comprised 13.79% of the total subjects. In group 2 (removable appliances), bleeding was absent in 27 (93.10%) subjects, while it was present in 2 subjects (6.90%). On Fisher's exact test, it was found that bleeding on probing on day 1 was similar in both groups and statistically nonsignificant (p = 0.670), as shown in Table 1.

Table 2 depicts the study groups according to the value of bleeding on probing obtained at 30 days. In group 1 (fixed appliances), bleeding was absent in 5 (17.24%), while it was present in 24 (82.76%) subjects. In group 2 (removable appliances), bleeding on probing was absent in 2 (6.904%) subjects, while it was present in 27 (93.10%) subjects. On Fisher's exact test, it was found that bleeding on probing at 30 days was similar in both groups and statistically nonsignificant (p = 0.423).

Table 3 represents the changes in the plaque index after insertion of fixed and removable appliances. In group 1 (fixed appliances), the mean value of change in the plaque index after insertion of fixed appliances was 0.1278 ± 0.1272. In group 2 (removable appliances), the mean value of change in the plaque index after insertion of removable appliances was 0.1547 ± 0.1207. It is evident that the mean plaque index value was slightly higher in group 2 (removable appliances) after 30 days of insertion, but on unpaired t-test, it was found that changes in the plaque index 1 month after insertion of both fixed and removable appliances were almost similar with a T value of 0.826 and was statistically nonsignificant with a p-value of 0.412.

Table 4 describes the changes in the gingival index after insertion of fixed and removable appliances. In group 1 (fixed appliances) bleeding on probing was absent in 2 (6.904%) subjects, while it was present in 27 (93.10%) subjects. On Fisher’s exact test, it was found that bleeding on probing at 30 days was similar in both groups and statistically nonsignificant (p = 0.423).

Table 5 describes the changes in the gingival index after insertion of fixed and removable appliances. In group 1 (fixed appliances) bleeding on probing was absent in 2 (6.904%) subjects, while it was present in 27 (93.10%) subjects. On Fisher’s exact test, it was found that bleeding on probing at 30 days was similar in both groups and statistically nonsignificant (p = 0.423).
appliances), the mean value of change in the gingival index after insertion of fixed appliances was $0.1633 \pm 0.1944$. In group 2 (removable appliances), the mean value of change in the gingival index after insertion of removable appliances was $0.652 \pm 0.1234$. It is evident that mean gingival index value was slightly higher in group 2 (removable appliances) after 30 days of insertion, but on the application of unpaired t-test, it was found that changes in the gingival index 1 month after insertion of both fixed and removable appliances were almost similar with a $T$ value of 0.044 and was statistically nonsignificant with a $p$-value of 0.965.

- **Table 5** shows the changes in the $S.\ mutans$ count after insertion of fixed and removable appliances. In group 1 (fixed appliances), the mean value of change in the $S.\ mutans$ count after insertion of fixed appliances was $1128 \pm 2048$. In group 2 (removable appliances), the mean value of change in the $S.\ mutans$ count after insertion of removable appliances was $146.6 \pm 376$. It is evident that the mean value of the $S.\ mutans$ count was higher in group 1 (fixed appliances) after 30 days of insertion. On application of unpaired t-test, it was found that changes in the $S.\ mutans$ count 1 month after insertion of both fixed and removable appliances were more in group 1 (fixed appliances) with a $T$ value of 2.538 and was statistically significant with a $p$-value of 0.014.

**Discussion**

Orthodontic treatment is becoming increasingly popular in today’s society for a variety of reasons, including the improvement of one’s appearance, mastication, and speech, as well as one’s level of comfort, self-esteem, and overall health. In the past, orthodontic treatment was primarily used to correct malocclusions.9 Orthodontic therapy makes it more difficult to maintain good oral hygiene, which enhances the accumulation of bacterial plaque. Gingival hyperplasia and bleeding on probing are common during orthodontic treatment. Deep probing depth spurred on by gingival hyperplasia may provide a favorable habitat for periodontopathogenic anaerobic bacteria.10 The frequency of biofilm formation and its problems have persisted despite several preventative measures used to reduce plaque formation on orthodontic appliances, particularly in youngsters and immune-compromised patients.11 The present study was conducted in an attempt to find out the effect of both fixed and removable intraoral appliances on oral health status and $S.\ mutans$ count.

To reduce inter-examiner bias, the patients were assessed by only one examiner during the whole trial. One month was deemed enough to determine the $S.\ mutans$ count and gingival health since prolonged examination might result in lower patient cooperation and erroneous findings. The age range employed in this research was 6 to 14 years. This age range was chosen because most preventative and interceptive orthodontic treatments may be completed at this period to address malocclusions and guide occlusion.12,13 The Silness and Loe plaque index and Loe and Silness gingival index14 are two of the most widely recognized plaque and gingival indices for assessment used in the study. The plaque and gingival indices of each subject were measured on the mesial, distal, buccal, and lingual/palatal surfaces of each tooth using a sterile periodontal probe according to Silness and Loe at baseline and after 4 weeks of insertion of the intraoral appliance.

Saliva samples were collected to test for $S.\ mutans$. Saliva samples were obtained from children, regardless of their gender, who met the set inclusion and exclusion criteria. The collection took place during the midday period at both baseline and one month later. To minimize the influence of circadian cycles and variations in unstimulated saliva flow rate, the samples were collected in sterile containers.15 Plaque and oral swab samples were not chosen for the detection of $S.\ mutans$ because all intraoral sites submerge their exudates in the saliva, which includes dislodged oral bacteria.16

Saliva samples were collected, transported to the laboratory, and processed within 30 minutes after collection to avoid molecular degradation. The MSB agar was commonly used as a medium for culture of $S.\ mutans$, like studies conducted by Lundström et al,17 Boyd,18 and Lee et al.19 Statistical analysis was done on the mean value of the plaque index, gingival index, bleeding on probing, and bacterial counts, and the data obtained were expressed as values of logarithm 10 (log 10) CFU. The results of the present study showed that there was no statistical difference from days 1 to 30 on bleeding on probing in both groups, that is, fixed and removable appliances. The above results obtained are in accordance with Zachrisson and Zachrisson20 who observed generalized moderate hyperplastic gingivitis within 1 to 2 months of placement of fixed orthodontic appliances.

There was statistically significant increase in the gingival and plaque indices in both groups from days 1 to 30. However, on comparing the gingival and plaque indices in both groups, the difference was found to be statistically insignificant. Sadowsky and BeGole21 evaluated the periodontal changes after fixed orthodontic treatment and found that the maxillary posterior and mandibular anterior regions of the mouth are generally affected. Sinclair et al22 also noted similar results and noted that mild gingivitis occurs on the labial surfaces of incisors after bonding of teeth.

Statistical analysis regarding the $S.\ mutans$ count in the present study showed a statistically significant increase after insertion of fixed appliances as well as removable appliances from days 1 to 30. But on comparison of both groups, it was found that the increase in the $S.\ mutans$ count was more in fixed appliances. The results of the present study were in accordance with the study by Kundu et al23 who suggested that the microscopic counts of $S.\ mutans$ increased significantly after the insertion of fixed and removable appliances.

Across the literature, studies have seldom been conducted to illustrate the effect of removable appliances on the periodontal health status and also there are very few studies comparing removable and fixed appliances. Arikan et al24 studied the effects of fixed and removable appliances on gingival and periodontal health and found an increase in both plaque and gingival scores, which is in agreement with the present study. In contradiction to present study, Türk­kahraman et al19 Peretz et al25 stated that fixed orthodontic appliances do not cause statistically significant changes in plaque scores. Arikan et al15 compared the plaque index,
gingival index, and bleeding on probing index after giving fixed and removable appliances and concluded that an increment was seen in both the groups but the fixed appliances group showed a higher score. Gurcan et al. studied the effect of fixed and removable space maintainer on dental plaque and dmft (decayed, missing, filled teeth) score and found an increase in the dmft scores in cases of removable space maintainers and band and loop space maintainers, while the dental plaque score was increased in cases on a lingual arch space maintainer. Hosseinipour et al. stated that bleeding on probing and gingival index increase following the placement of fixed and removable space maintainers.

The limitation in this present study is that these gingival and periodontal tissue changes are reversible on removal of appliances, which was not evaluated. Also, the follow-up period in the present study is only 1 month. Further studies with a longer follow-up period are required.

**Conclusion**

Fixed and removable orthodontic therapy is a necessity to correct malocclusion, although it has been observed that it leads to an increased level of plaque and creates difficulty in maintaining oral hygiene. It is imperative to deliver proper oral hygiene instructions while giving the appliances.

On the basis of the results obtained in this study, the following conclusions could be drawn:

- Plaque and gingival scores increase after insertion of both fixed and removable appliances.
- Fixed orthodontic appliances significantly lead to an increase in the S. mutans counts in the oral cavity.

The inherent levels of these microorganisms are related with plaque accumulation due to fixed or removable appliances. Increase in the level of plaque and microorganisms after fixed and removable appliance therapy can hamper optimum oral health and thus is a concern for pediatricians.

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None.

**Conflict of Interest**

None declared.

**References**