Clinical and Experimental Health Sciences

Salivary pH Changes and Biofilm Formation During Active Orthodontic Treatment with Clear Aligners and Fixed Appliancese

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ABSTRACT

Objective: To examine salivary pH changes and the plaque formation in two different orthodontic treatments clinically.

Methods: The study sample included 40 patients, who were divided into two groups according to the type of orthodontic appliance: Group CA, (n=15) clear aligners; Group FT, (n=25) fixed appliances. Group FT received both metal (FT/SS) and elastic ligatures (FT/EM) for 2 weeks respectively to test the effect of ligature type also. Salivary pH values, plaque index, and plaque percentage were measured at T0 (after scaling and polishing) and T1 (after 2 weeks). pH was measured with a digital caliper (HI 2211 pH/ORP Meter) and plaque was identified by a discoloring agent (Tri Plaque ID Gel). The Paired t-test, Independent t-test, Anova test and Pearson Correlation tests were used in the statistical analysis.

Results: There was significant decrease in salivary pH values after two weeks of metal ligature in FT/SS. Plaque index and plaque percentage parameters showed significant incremental changes between groups with the least increase in CA, followed by FT/SS and FT/EM respectively.

Conclusion: Different types of orthodontic treatment and ligatures significantly effected salivary pH and the amount of plaque formation during orthodontic treatment. Aligners had the least effect on salivary pH and plaque formation while fixed treatment with elastic ligature affected the most. Therefore, aligner treatment may be more beneficial for patients with compromised oral hygiene.

Keywords: Orthodontic appliances, corrective orthodontics, saliva, dental plaque

1. INTRODUCTION

Orthodontic treatment has become increasingly popular in both adolescent and adult patients (1). Fixed appliances constitute the conventional treatment method in orthodontics to restore esthetics and function (2). However fixed appliances can impede brushing and decrease self-cleansing by the saliva, mastication and tongue (1,3). This consequently results in plaque accumulation, which impairs gingival health (4). It has been reported that comprehensive orthodontic treatment on average requires a mean treatment time of 20 months, with a wide range of treatment durations such as 14-33 months (5). Excessive treatment duration and fixed appliances have been associated with a greater susceptibility to adverse effects, including root resorption (6) and plaque-induced conditions, primarily demineralization (7) and microbial changes (8). Therefore, it has been recommended for clinicians to consider the effects that orthodontic treatment, including appliance type, may have on periodontal health (9).

Clear aligners (CA) are removable orthodontic appliances, which are considered as esthetic and comfortable alternatives to fixed treatment (10). Their removable nature makes it easier to maintain dental hygiene than fixed appliances (11). In order to prevent periodontitis, clear aligners have been recommended in orthodontic treatment plans (11-14) and they have been linked to better periodontal health and lower levels of periodontopathic bacteria (11). However, some investigators emphasize that it is important to have a sound judgment regarding the periodontal effects of clear aligners considering that they cover teeth and keratinized gingiva for most of the day (12,15).

Whole saliva, which contains oral germs and food particles, is a complex mixture of fluids produced by the major and minor salivary glands as well as the gingival cervical fluid (16). pH is one of the qualitative properties of saliva (17). Along with other qualitative (salivary protein content, viscosity and buffer capacity) and quantitative properties (the flow rate), salivary pH aid in the equilibrium between demineralization and demineralization of enamel (17). The average pH of whole, unstimulated saliva is typically between 6.75 and 7.25, controlling the pH of the majority of oral surfaces (16).

Dental plaque is a highly complex organization in a biofilm form (1) and is considered the main causative factor in dental caries and periodontal disease (1,18). The pH of plaque is about 6.7. Enamel demineralization occurs when the pH falls below 5.5, which is considered essential (19). This decrease aids in the

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development of white spot lesions, which are said to affect 50% of orthodontic patients (20). Streptococcus mutans and Lactobacilli, two bacteria that produce acid, are the primary produced colonies of particular interest (21). It has been found that the type of orthodontic materials such as the type of archwire ligation material has an impact on plaque harboring surrounding the brackets (21,22). According to most studies, stainless steel (SS) ligatures showed less plaque retention than elastomeric modules (EM) whereas; higher concentrations of acidogenic bacteria can be found with EM ligatures, most notably Streptococcus mutans and Lactobacilli (3,22,23).

The aim of this study was to assess changes on biofilm accumulation and oral cavity pH in different treatment modalities (clear aligners and fixed appliances with metal or elastic ligation) during active orthodontic treatment, an aspect which has not been studied previously; under the null hypothesis that there were no significant differences between different orthodontic appliances on plaque accumulation and saliva pH during active treatment.

2. METHODS

This randomized, cross-sectional clinical trial was approved by the Marmara University Clinical Research Ethics Committee, on 21.02.2022 and with the number 09.2022.297. Two groups of active orthodontic treatment patients who have been under treatment for at least three months in the clinic of Orthodontic Department, Collage of Dentistry, Marmara University, were selected randomly from the active treatment patients' list. All patients or their guardians had provided signed informed consent forms. It was calculated that to have 80% power to detect an effect size, it would be sufficient to have a total sample size of n = 30.

Inclusion criteria were: having non-extraction orthodontic treatment either with fixed appliances or clear aligner treatment for 3-6 months, skeletal and dental Class I malocclusion (SNA: 82°, SNB: 80°) with mild to moderate crowding (3-7 mm) and normal vertical growth pattern (mandibular plane angle: 25°, sum of inner angles: 396°, maxillary height: 60°), age between 16-24 years, orthognathic profile with lip competency, good oral hygiene, no drug usage, same brand and series of brackets in fixed treatment, same manufacturer in clear aligner treatment, permanent dentition. Exclusion criteria were: having less than three months of treatment, single arch undergoing orthodontic treatment, having additional palatal or lingual appliances or attachments (i.e. hyrax screw, transpalatal arch, lingual button etc.), presence of systemic diseases or mouth-breathing, pregnancy, smoking, poor oral hygiene, interrupt or discontinue treatment, previous orthodontic treatment, crown restorations, active periodontal disease or caries. Also, for CA group, all subjects had a minimum of 9 attachments per jaw between 1st molars. 2nd molars were omitted in this regard since FT patients were also bonded between the 1st molars.

The clear aligner group (CA) included 15 aligner orthodontic treatment patients (9 males and 6 females; mean age 17.5 years). The fixed treatment group (FT), contained 25 fixed orthodontic treatment patients (13 males and 12 females; mean age 18.2 years) who went through 2 phases during study: metal ligation for 2 weeks and elastomeric ligation for 2 weeks, respectively. Demographic characteristics of the patients are shown in Table 1.

***Table 1**. Demographic characteristics of the enrolled patients*

Characteristics		CA	F FT	Total
Number of subjects (n)		15	25	40
Gender [A]	Male	9 (60%)	13 (52%)	22 (55%)
	Female	6 (40%)	12 (48%)	18 (45%)
Age (Years) [B]	Male	17.9	17.6	17.7
	Female	16.9	18.8	18.2
Total		17.5	18.2	17.9
pH measurement at T0 [B]		7.08 ± 0.29	7.18 ± 0.27	7.13 ± 0.28
Plaque index and percentage		00	00	00

CA: Clear Aligners; FT: Fixed treatment; [A]: Qualitative data expressed as frequency and percentage. [B]: Continuous data expressed as mean ± standard deviation

Prior to treatment, all patients were referred to Periodontology Department and underwent meticulous phase 1 periodontal therapy. At this stage, patients received oral hygiene instructions. Additionally, at the bonding appointment, all patients received a second instructive session on how to maintain good oral hygiene with orthodontic appliances. During orthodontic treatment, two investigators observed all patients and confirmed that subjects had acceptable oral hygiene habits throughout.

At the time of measurements, patients were asked not drink or eat anything except water over the night until their morning appointment. Periodontal scaling and polishing (Prophylaxis) was done. Tri Plaque ID Gel (GC Corporation, Tokyo, Japan) was used to identify all plaque areas. Scaling was done with ultrasonic and hand instruments to make sure that all the plaque has been removed and the plaque score was made 0 by the same researcher. In proximity of the bonded attachments or brackets, additional care was taken in order not to cause debonding. Hand instruments were preferred in these areas. Prophy cups and brushes were also used. As T0 measurements, salivary pH, plaque index and plaque percentage were recorded. 2 weeks after (T1), patients were asked to come again for the follow up measurements.

At both time-points, firstly the unstimulated saliva samples were collected in the morning between 9 A.M. and 12 P.M. Before taking the saliva sample, patients were asked to rinse their mouth with distilled water and asked to swallow the remnants till they felt their mouth dry. Then the patients pooled the unstimulated saliva in their mouth for 2 minutes, and were asked to drool 5 ml of the pooled saliva passively in 10 ml plastic lab tube. Saliva pH was measured with HI 2211 pH/ORP Meter (Hanna Instruments Inc, USA) with PH Probe Composite Electrode (Elprico, Shenzhen, China). The probe sensor was allowed to equilibrate with the environment before each measurement and was rinsed with distilled water spray and wiped dry gently. pH 4 and pH 7 buffering agents

were used for calibration. Samples were measured in the same order of the appointments. Between each sample, the pH probe was rinsed with distilled water and wiped gently to dry.

After the saliva sample has been collected, Tri Plaque ID Gel was applied on the distal, labial and mesial surfaces from tooth #17 to tooth #47. Patients were asked to rinse their mouth for 2 minutes for the discoloring gel to color the biofilm formed on the teeth surfaces. At T0, the prophylaxis procedure provided that the plaque scores were set 0 as the baseline. At T1, plaque index scores were given depending on the plaque mean index standards: 0, meaning there is no plaque; 1: there is biofilm but a very shallow amount of immature plaque that was formed newly in the past 48 hours and the gel dyes it in pink color; 2: moderate amount of biofilm which the gel dyes in purple color; and 3: mature thick biofilm identified as the acidic plaque pH (< pH 4,5) that has the tendency of enamel demineralization and caries formation, gel dyes it in light blue color. These scores were determined by two investigators and recorded on a periodontal chart and then transferred to an excel sheet and photos were taken. For plaque percentage measurements, the total number of teeth surfaces that presented plaque was divided over to total number of teeth surfaces that were examined. These percentages were also noted in the excel data sheet.

In FT group, first phase included the T0 and T1 measurements with 0.010-inch stainless steel ligatures placed (FT/SS). The second phase included prophylaxis, T0 and T1 measurements with elastomeric ligatures placed in the same patient group (FT/EM). All FT patients had nickel titanium wires with 0.022x0.025-inch slot brackets (Rocky Mountain Orthodontics, Franklin, USA) for more standardization. At the end of the study patients were shown the areas that they need to clean more properly; oral hygiene instructions were elaborated.

Statistical Analysis

The data was analyzed using IBM SPSS (Statistical Package of Social Sciences; IBM Corp, NY, USA) software for Windows, version 28.0.

Statistical methods were used to analyze the data, including the calculation of descriptive statistics such as the frequency and percentage for categorical variables, and the mean, the standard deviation (SD), and the minimum and maximum for the continuous variables.

The Shapiro-Wilk test of normality was applied to evaluate the normal distribution of the parameters, and since the data was found to support parametric assumptions, a paired-samples t-test was performed to compare between the before and after scaling and polishing with two weeks of orthodontic treatment measurements for the same group of patients in fixed group and for patients with aligner treatments. Additionally, the paired t-test was performed to compare the measurements between T0 and T1. Pearson correlation test was performed to evaluate the associations between specific corresponding variables. ANOVA test was performed to evaluate if different types of orthodontic treatment have a significant effect on the pH change and plaque accumulation. An alpha level of .05 was used for all statistical tests and all were two-tailed.

3. RESULTS

The total sample was 40 patients. The majority of the sample was male (n=22, 55%) (Table 1).

In the CA group, the pH change was insignificant at T1-T0 ($p > .05$); while a statistically significant increase in the mean of plaque index and plaque percentage values ($p < .001$) were observed (Table 2). At T1, the highest dental plaque accumulation was observed at the upper right quadrant whereas; the least plaque accumulation was on the lower left quadrant (Figure 1).

***Table 2**. Evaluation of the changes in Clear Aligner (CA) group*

	T0	T1	Mean Difference	P value#
	Mean ± SD	Mean ± SD		
pH	7.081 ± 0.286	7.028 ± 0.332	0.053	.575
Plaque index	00 ± 00	0.205 ± 0.103	-0.205	.00*
Plaque percentage	00 ± 00	20.509± 10.383	-20.509	.00*

*Paired-samples t-test. *: The mean difference is significant at the .05 level; SD: standard deviation.*

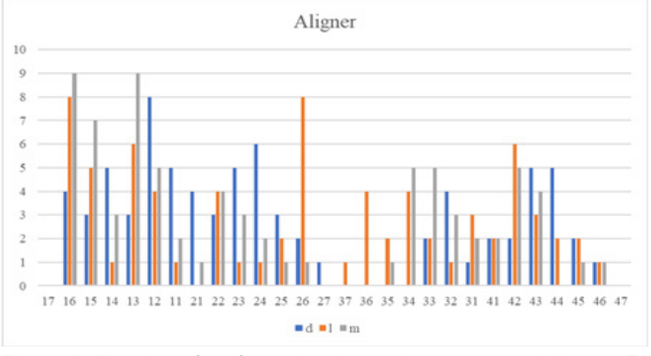

***Figure 1.** Number of surfaces that have plaque accumulation at T1 of CA. d: distal, l: labial and m: mesial.*

For the FT group; FT/SS showed a statistically significant decrease in the mean pH value ($p < .05$). At T1, plaque index and plaque percentage means were significantly higher than the mean at T0 as shown in Table 3. The highest dental plaque accumulation was observed on the labial/buccal tooth surfaces at T1 (Figure 2).

***Table 3.** Evaluation of the changes in FT/SS group*

	T0	T1	Mean Difference	P value#
	Mean ± SD	Mean ± SD		
pH	7.192 ± 0.312	6.956 ± 0.351	0.236	.027*
Plaque index	00 ± 00	0.481± 0.151	-0.481	.00*
Plaque percentage	00 ± 00	41.104± 10.946	-41.104	.00*

*Paired-samples t-test. *: The mean difference is significant at the .05 level; SD: standard deviation.*

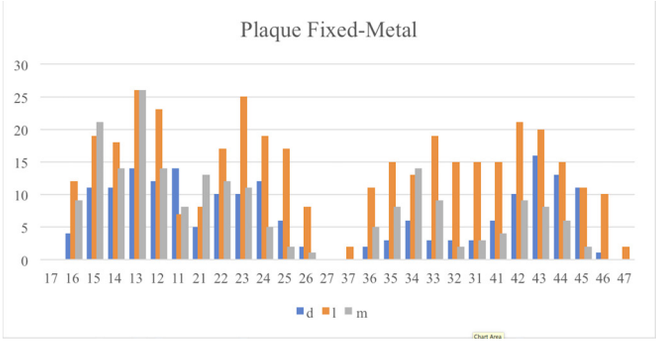

***Figure 2**. Number of surfaces that have plaque accumulation at T1 of FT/SS. d: distal, l: labial and m: mesial.*

In FT/EM group, there was a statistically insignificant decrease in the mean pH value ($p > .05$). The plaque index and plaque percentage means were significantly higher at T1 than the mean at T0 ($p < .001$) (Table 4). Similar to FT/SS, the highest dental plaque accumulation was observed on the labial/buccal tooth surfaces at T1 (Figure 3).

***Table 4.** Evaluation of the changes in FT/EM group*

	T0	T1	Mean Difference	P value#
	Mean ± SD	Mean ± SD		
pH	7.166± 0.230	7.054± 0.341	0.112	.143
plaque index	00 ± 00	0.610± 0.149	-0.610	.00*
plaque percentage	00 ± 00	55.886± 11.729	-55.886	.00*

*Paired-samples t-test. *: The mean difference is significant at the .05 level. SD: standard deviation.*

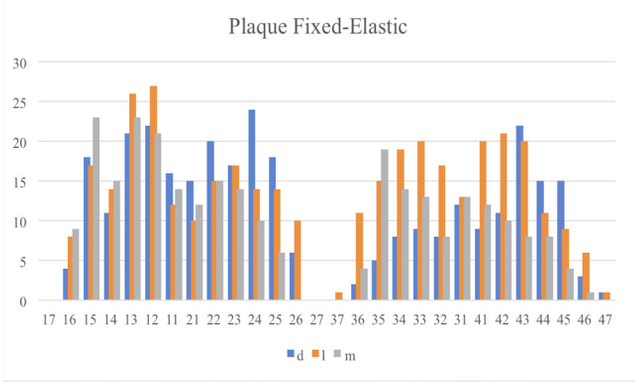

***Figure 3.** Number of surfaces that have plaque accumulation at T1 of FT/EM. d: distal, l= labial and m= mesial.*

Intergroup comparisons revealed a significant difference between all groups in plaque index and percentage parameters (Table 5). For the salivary pH, a significant difference was found between the means of pH change between CA and FT/SS groups, FT/SS and FT/EM groups; while insignificant difference was found between CA and FT/EM groups.

***Table 5.** Intergroup comparisons for all parameters.*

Parameters	Groups	P value		
		CA	FT/SS	FT/EM
PH	CA		.0004	.527
	FT/SS	.0004		.0001
	FT/EM	.527	.0001	
Plaque index	CA		.0005	.0001
	FT/SS	.0005		.0001
	FT/EM	.0001	.0001	
Plaque percentage	CA		.0002	.0006
	FT/SS	.0002		.0001
	FT/EM	.0006	.0001	

CA: Clear aligner; FT/SS: Fixed treatment with stainless steel ligation; FT/EM: Fixed treatment with elastomeric module ligation

Plaque index and pH correlations within groups are shown in Table 6. In all groups, the two parameters were found to be correlated with a weak negative relation and the correlations were statistically insignificant.

***Table 6.** pH and plaque index correlations in three groups.*

		Plaque index		
		CA	FT/SS	FT/EM
pH	Pearson Correlation Coefficient	-0.322	-0.168	-0.36
	P value	.242	.421	.255

CA: Clear aligner; FT/SS: Fixed treatment with stainless steel ligation; FT/EM: Fixed treatment with elastomeric module ligation

4. DISCUSSION

Orthodontic patients go through different kinds and methods of treatments that have the same goal of improving the patients' esthetics and functions by reaching the ideal occlusal relationships and alignment (1). As clinicians, we needed to consider the side effects of the orthodontic treatment that may impair treatment outcome and the patient's oral health (1,6-9). Decrease in the salivary pH and biofilm formation can lead to serious oral health problems like dental caries and periodontal tissue inflammations, which lead to shortening in the tooth longevity and esthetics (1).

Primary aim of this randomized clinical study was to evaluate the changes that happen in the oral cavity (regarding salivary pH and biofilm formation) during active treatment with different kinds of orthodontic appliances: clear aligners and fixed appliances with two ligation methods (elastic and stainless steel). The secondary aim was to assess if changes in oral biofilm formation were associated with the change of salivary pH. Several aspects of orthodontic appliances on oral environment such as their impact on tooth wear (19), demineralization (20), oral microbiome (21-23), salivary properties (24,25) have been assessed in a number of earlier researches. However, the impact of the present three kinds of orthodontic materials on salivary pH and biofilm development has not been examined previously.

In the present study, plaque index and plaque percentage parameters showed significant incremental changes between groups with the least increase in CA, followed by FT/SS and FT/EM respectively. However, pH values did not show a parallel increment with the plaque parameters and there was no significant correlation between pH and plaque accumulation. Actually, the pH values that were obtained at both time points can be considered within the average range (pH 6.2-7.6) of saliva (26). This finding can be a result of the study design. Although initial pH values were measured after a prophylaxis application, patients were already undergoing the treatment at that time-point. And at the second measurement, patients were still undergoing the treatment. The change in pH could be more significant if the pretreatment pH values were available to evaluate the effect of treatment modality on the salivary pH in a prospective study design. Therefore, it can be stated that the pH of the unstimulated saliva was independent of plaque accumulation parameters while undergoing treatment. Several investigators reported similar results to our findings regarding the salivary pH and stated that salivary pH did not significantly change between the studied time points during fixed orthodontic treatment (27-29).

However, there is a lack of consensus on this issue. Alshahrani et al (25) evaluated the alterations in salivary parameters prospectively in patients undergoing fixed orthodontic therapy. They found a significant reduction in salivary pH, total protein concentration, and calcium level in saliva of fixed orthodontic appliances group. On the contrary, Chang et al (30) reported a significant increase in stimulated salivary pH, flow rate, buffer capacity, plaque index scores, and in the levels of streptococcus mutans and lactobacilli after three months of active treatment. However, these studies did not specify the type of ligation material. Al-Haifi et al (31) compared the short-term effect of SS and EM ligatures and concluded that EM ligatures showed a significant decrease in salivary pH. This result was contradictory to our findings, where the SS group decreased more than EM. These studies differ from the present study in methodology, since they compared the pH values of before and after treatment in a prospective design. Another contributory factor in this difference can be the limitations of salivary pH as a diagnostic bio-meter. Several uncontrolled factors such as the diet, lifestyle, and salivary flow rate can affect its value (26). In this study, these factors were aimed to be controlled by having the values of T0 and T1 from each patient at the same hour of the day, without any influence on their lifestyle or diets. Patients were asked not to eat or drink anything before the appointment overnight, and unstimulated (resting) whole salivary samples were collected in a separate room with a quite environment to prevent mechanical or chemical stimuli as in previous studies (24,25).

The increase in plaque accumulation is a direct consequence of impeded oral hygiene procedures (13). Plaque accumulation can favor the transition of the microbial biofilm to a more aggressive periodontopathogenic flora (1). The current findings regarding plaque index and percentage are in accordance with the literature (13,14). A meta-analysis by Jiang et al (14) compared periodontal health in patients undergoing orthodontic treatment with clear aligners with that of those undergoing orthodontic treatment with fixed appliances. They concluded that clear aligners were better for periodontal health, including plaque index, gingival index, and probing depth than were fixed appliances (14). This expected result can be explained by the removable nature of CA, which provides an ease of access to dental and interdental surfaces during brushing (13). Also, retentive areas are much less in CA compared to fixed appliances for two reasons. Firstly, not all teeth receive attachments; in many cases one or two teeth can be free of them. In the present group, all posterior teeth including 1st molars and the premolars had attachments mainly for retentive purposes and for premolar rotations while one or two of anterior teeth did not require attachment placement. Secondly, their surface designs are either convex or flat, whereas brackets have indentations, concavities and undercuts (13).

Regarding SS and EM ligatures, the differences in surface topography, organic content and inertness of these materials are considered as factors that cause different bacterial colonization patterns (32); which is enhanced in EM (3,22,23) due to its organic and porous surface. EM ligature materials are thicker in dimension; therefore, blocking the teeth surface more and creating narrow areas that cannot be cleaned. The current findings are consistent with those of Forsberg et al (23), who found that EM ligatures had greater levels biofilm formation. They also reported increased Streptococcus mutans and Lactobacillus colonization with EM than SS ligatures and recommended to avoid using EM on individuals who did not maintain good dental hygiene. Turkkahraman et al (3) reported that elastomeric rings were more likely to cause bleeding than steel ligatures, but they did not find any appreciable differences in bleeding upon probing or plaque index values. On the other hand, Souza et al (33) related elastomeric rings, as opposed to steel ligatures, to higher scores for bleeding on probing and plaque index. These results indicate that patients treated with fixed appliances are more likely susceptible to gingival inflammation (13).

As a general observation, it was noticeable that the first quadrant of the mouth (upper right) had the most plaque accumulation, concentrated between the lateral incisor and canine area. But the teeth that had most plaque accumulated on its surfaces was the right maxillary first molar, which is due to the reason that most of the sample size were right-handed people and they could not use the correct brushing technique due to the difficulty of accessing the area. Lower left quadrant had the least biofilm formation, which can be explained by contentious salivary flush plus the muscles movements during speech and mastication providing as a physical rub for the buccal surfaces of the teeth in addition to the easier access for tooth brushes and other oral hygiene instruments. Despite that, still plaque accumulated on the buccal surfaces of these teeth. The short follow-up period and limited number of subjects were limitations of this study. A longer observational time in a larger sample and including other periodontal indices can be more informative for an evaluation of the periodontal outcomes of orthodontic

treatment modalities. Another limitation can be considered as the lack of attachment standardization in CA group. It is not possible to have same attachments on the same teeth in all aligner patients due to the nature of orthodontic therapy with aligners. These cases were designed uniquely, according to the individual needs of the malocclusion. However, this issue was tried to be controlled by the inclusion criteria. All subjects in CA group had attachments on a minimum of 9 teeth per jaw.

Orthodontic therapy is specific to patient. Both fixed and removable appliances are vital tools of orthodontic treatment. Each material has its specific properties, designed to achieve the primary goals of orthodontic therapy; while the clinician holds the priority to choose the most beneficial modality for the oral and general wellbeing of the patient. Clinicians should take periodontal effects of appliances into consideration while making this choice, especially in compromised cases.

5. CONCLUSION

The null hypothesis was rejected. The type of orthodontic materials affected the mean plaque index and percentage. The aligner group had the least levels of plaque index, followed by fixed treatment with SS and EM ligatures respectively. Orthodontic treatment with clear aligners can be more beneficial for periodontally compromised patients.

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Design of the study: YYMAE, YBA
Acquisition of data for the study: YYMAE
Analysis of data for the study: YYMAE
Interpretation of data for the study: YYMAE, YBA
Drafting the manuscript: YYMAE, YBA
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