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Comparison of Plaque Retention in Different Commercially Available Clear Aligners Using An ATP Bio-Luminometer

Shubhita Sethi [1], Shailesh Deshmukh[2], Veera Bhosale[2], Priya Deo[3], Siddharth Shinde[2] ,Simran Sharma[1]

1 - Post Graduate, Department of Orthodontics and Dentofacial Orthopedics, Bharati Vidyapeeth Dental College & Hospital, Pune, Maharashtra, India.

2 – Faculty, Department of Orthodontics and Dentofacial Orthopedics, Bharati Vidyapeeth Dental College & Hospital, Pune, Maharashtra, India.

3 – Faculty, Department of Oral and Maxillofacial Pathology and Oral Microbiology, Bharati Vidyapeeth Dental College & Hospital, Pune, Maharashtra, India.

Corresponding Author: Dr Siddharth Shinde, Assistant Professor, Department of Orthodontics, Bharati Vidyapeeth (Deemed to be University) Dental College & Hospital, Pune, Maharashtra, India. Phone no: +91 9766000500, Email: siddharth8785@gmail.com

ABSTRACT

Objective : The study was designed to compare 4 different commercially available brands of clear aligners based on their plaque retention properties after 14 days of wear.

Methods: 26 aligners were evaluated under each brand, making it a total of 104 aligners. ATP Bio-luminescence was used to quantify the microbial plaque adherent to the inner surface of aligners after being thoroughly rinsed, dried and cleaned. The patients were also asked to fill out an oral hygiene proforma, to eliminate the subjective factors. to remove any patient subjectivity (rewrite).

Results: The plaque retention on different brands of clear aligners after 14 days of wear showed no statistically significant difference. However, a positive correlation was observed between oral hygiene habits such as frequency of brushing and mouthwash, habits's remove) frequency of consumption of sugar added drinks and plaque retention on the aligner surface.

Conclusion: The 4 brands of commercially available clear aligners studied were highly comparable with no significant difference in terms of plaque retention.

Keywords: Plaque retention, Clear Aligners, Aligners, ATP Bio-Luminometer.

INTRODUCTION

In an attempt to find a more hygienic and aesthetic alternative to orthodontic bands and brackets, clear aligners have revolutionized the field of orthodontics. They have not only offered an advantage on the aesthetic front but have also proven to facilitate good oral hygiene [(1,2)] . With the advantage of being able to remove the aligner and clean the tooth surface appropriately, clear aligners have tremendously improved oral hygiene conditions with orthodontic treatment [(3)]. However, despite these advantages, various case reports have described instances of enamel demineralization, decalcification, and increased instances of dental caries in patients undergoing orthodontic treatment with clear aligners [(4)] .

Clear aligners cover the overall surfaces of teeth and gingiva, i.e., the palatal, lingual, labial, buccal, occlusal, and incisal surfaces. This is seen to have a direct influence on the oral microbial flora and plaque retention as they prevent the natural cleansing of teeth by obstructing the flushing, buffering, and remineralizing effect of saliva and mucous tissues. Moreover, by interrupting the usual cleansing action of the tongue, lips, and cheeks they allow increased entrapment of food and dental plaque under the aligner surface leading to increased demineralization and decalcification [(4)].

Dental plaque which is primarily composed of bacteria in a matrix of salivary glycoproteins and extracellular polysaccharides adheres tenaciously to the intraoral hard surfaces, including removable and fixed restorations. The location of plaque in the oral cavity is significantly associated with diseases of the periodontium. While marginal plaque, is seen to have a high significance in the initiation and development of gingivitis, supragingival plaque and tooth-related subgingival plaque are considered vital in calculus formation and root caries. Similarly, tissue-related subgingival plaque plays an important role in tissue destruction that is visualised as different forms of periodontitis.[5] Plaque with orthodontics has resulted in various instances of enamel demineralization and white spot lesions [(6,7)] .

ATP (Adenosine Triphosphate) is a chemical substance acting as an energy source for all living organisms. The presence of ATP is considered as proof of the presence of a living organism or a substance produced by the organism. ATP measurement was developed to estimate bacterial cell numbers in biological samples to ensure the microbiological quality. ATP bioluminescence reaction is one in which ATP is enzymatically consumed to produce light. Specifically, in the presence of the substrate luciferin, the enzyme luciferase uses the energy from ATP to oxidise luciferin and release photons (light at a wavelength of 562 nanometres). These released photons are then detected and measured by a luminometer, that is equipped with a photomultiplier tube, accurately quantifying the ATP present [(8)]. ATP bioluminescence being a highly rapid, accurate and non-invasive technique has slowly been gaining immense popularity

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in the field of dentistry to detect plaque accumulation and microbial viability [9-13].

While Invisalign (Align Technology, California) stands to be a pioneer in the field of clear aligners, various other brands of clear aligners have recently come up in the market, providing orthodontic treatment through clear aligner therapy. Compared to Invisalign, not much is known about the materials, properties, and reliability of these clear aligners. Even though studies have been conducted comparing various mechanical properties of available clear aligners and thermoplastic materials, very little data is available on plaque retention of different clear aligners [14-18]. Among the different properties, plaque retention of different clear aligners is important to evaluate as not only does it have a direct impact on the oral hygiene and conditions of the patients but also very minimal information is present regarding the different clear aligners present in the market.

So, the aim of this study was to evaluate and compare the plaque retention of different commercially available clear aligners available in the Indian market after 14 days of aligner wear. This is the first orthodontic study to compare the hygienic effects of different commercially available clear aligners after being thermoformed and using ATP bioluminescence in the quantitative evaluation of microbes on the aligner surfaces.

MATERIALS AND METHODS

This was a prospective clinical study approved by the Institutional Ethics Committee of Bharati Vidyapeeth Deemed to be University Dental College and Hospital, Pune (ECR/328/Inst/MH/2019). The study involved the comparison of plaque retention on four different leading brands of clear aligners commercially available in the Indian market using an ATP bio-luminometer.

Sample size determination: Keeping the level of significance at 5%, the sample size (n) was calculated using the formula: n=[(Z1-α-Zβ) σ]2/L2. Using the above formula, a minimum sample size per group derived was 26 aligners, considering 4 groups; a total sample size of 104 aligners was derived.

The inclusion criteria included (1) patients above 13 years of age, (2) patients with good oral hygiene, and (3) patients with good periodontal health. All patients below the age of 13 years, syndromic patients, patients with poor oral hygiene or periodontal health and those on medications that caused xerostomia were excluded from the study. To avoid any legal issues, the brands of aligners assessed in the study were labelled as Aligner Brand 1, Aligner Brand 2, Aligner Brand 3, and Aligner Brand 4.

The HY-Lite 2 System (Merck, Millipore, Germany) ATP bio- luminometer was procured for the study to quantify the amount of plaque bacteria adherent to the surface of different clear aligners. It is a portable machine with 11 x 13 x 28 cm in dimensions and 1.3kg **(Fig.1).** It has a working range of 0 – 99000 Relative Light Units (RLU). It allows accurate quantification of ATP present on a surface by precisely measuring the light released in a highly specific biochemical reaction:

$$ATP + LUCIFERASE\ REAGENT \rightarrow AMP + PP + LIGHT(h\nu)$$

The light emitted in the chemical reaction is a by-product, the intensity of which is indicated on the display of the machine in RLU. The RLU value is directly proportional to the quantity of ATP in the sample tested and thus directly proportional to the amount of plaque or microbes retained on the surface of the clear aligners.

Figure 1: HY-Lite 2 ATP Bio luminometer (Merck, Germany) (a) Frontal view, (b) Lateral view, (c) Back view showing attachment points. (d) Arial view

A ready-to-use test cuvette for use in a HY-Lite luminometer is the HY-Lite surface testing pen (Merck, Millipore, Germany). It is made up of the following sections: (1) A rinse solution that has been desegregated with a protective cap. (2) A white sampling stick (protected by a cap) for taking exact samples of the liquid being examined. An extractant is applied to the stick, which releases ATP from cellular material. The stick was also used to transfer the sample into the cuvette and, in a later stage, to open the reagent chamber. (3) Dilution, buffering, and neutralisation of the sample in a cuvette filled with test buffer. (4) An aluminium-foil-sealed reagent compartment containing a freeze-dried and stabilised luciferin/luciferase mixture as seen in Fig 2.

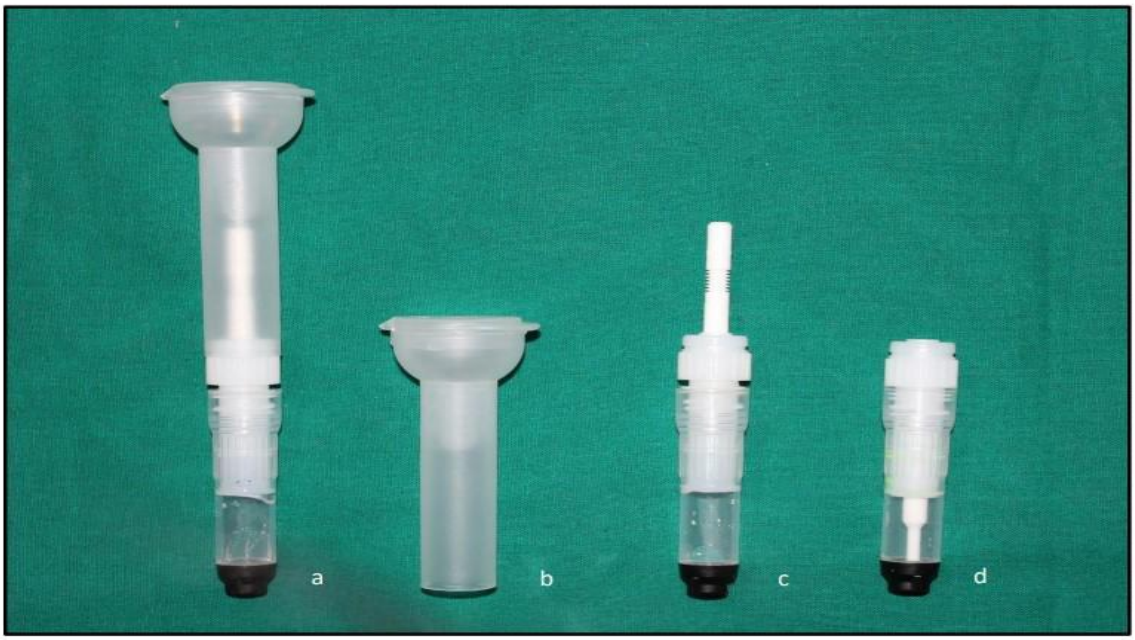

Figure 2: HY-Lite 2 Surface Testing Pens. (a) An unused surface testing pen on the left and a used pen on the right. (b)- Protection cap with integrated rinse solution, (c)- White sampling stick, (d)- Cuvette filled with test buffer and Reagent chamber sealed with aluminium foil, containing freeze-dried and stabilized luciferin/luciferase mixture.

The aligners the patients had been wearing for the last 14 days on their visit to the dental clinic were used for evaluation of plaque retention.

The patients were asked to remove the upper and lower aligners from their mouths and place them directly into the instrument tray. The aligners were then rinsed rigorously under tap water followed by mild washing with sterile water. The aligners were then allowed to dry thoroughly with air under pressure. Sealed swab sticks and HY-Lite 2 surface testing pens were used for testing each aligner. The swab stick was removed and dipped into the testing liquid present in the top chamber of the ATP testing pens**.** The wet swab stick was then used to swab the inner surface of the clear aligners in a circular motion from the right molar region to the left molar region, with circular swabs at each tooth depression in the aligner. The swab stick with all the ATP around it was then again dipped into the rinse solution.

The swab stick was vigorously rotated between the index finger and the thumb for about 10 seconds, to wash out the sample into the rinse solution**.** The pen was then carefully removed from the protection cap and the white sampling stick was dipped completely into the rinse solution for about 3 seconds**.** The stick was then pressed vertically under constant pressure against the swab stick or aligner surface.

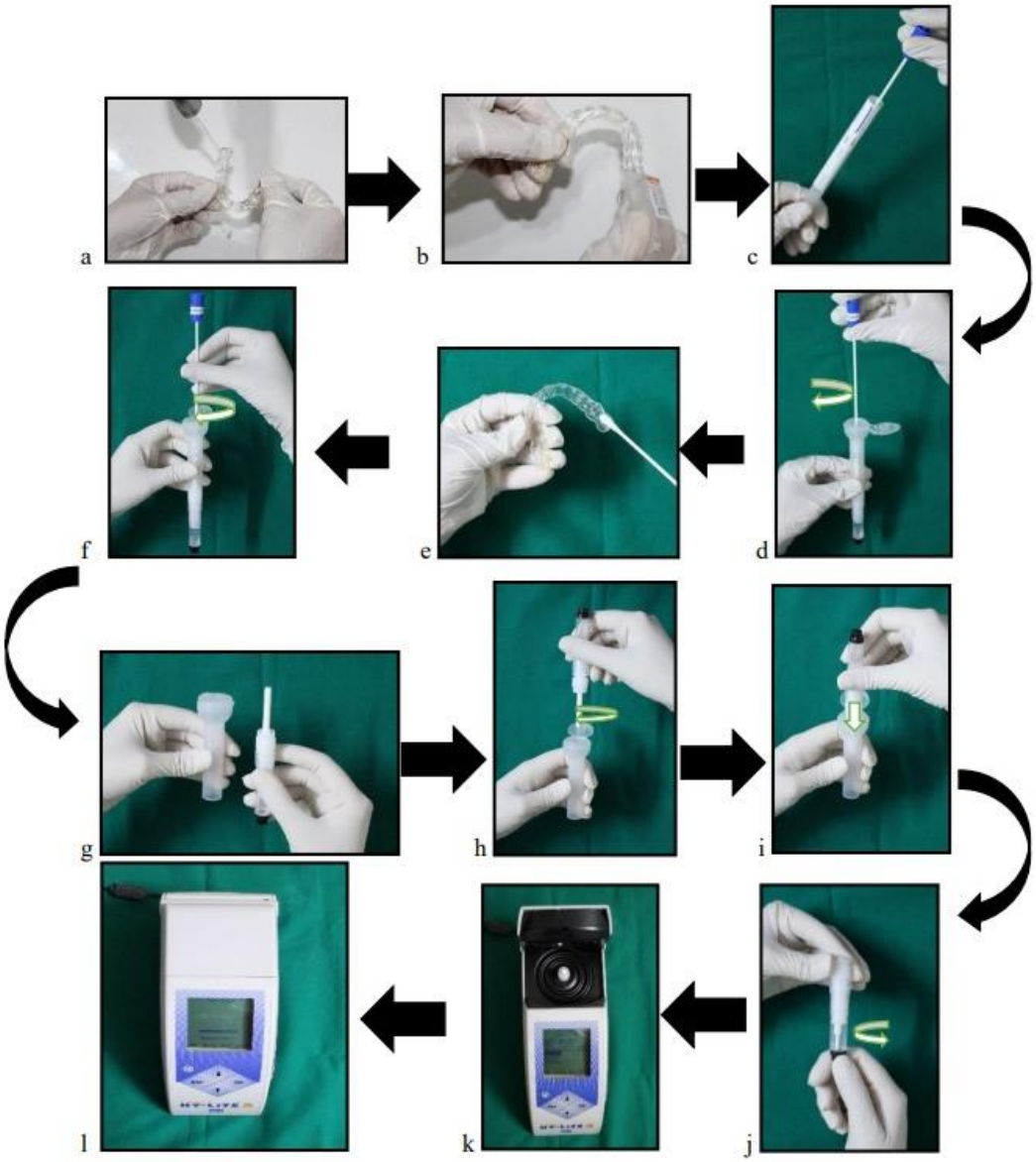

Figure 3: Steps of Data Collection. (a)- washing of aligners under tap water. (b)-Rinsing with sterile water. (c)-Removal of the sealed swab stick. (d)-Dipping the swab stick in the test liquid of the ATP testing Pen. (e)-Swabbing of the aligner. (f)- Washing out the sample into the test liquid. (g)-Testing pen removed from the protection cap. (h)- Testing pen dipped into the testing solution. (i)- testing pen pressed down under constant pressure. (j)-the pen was then turned to tear open the aluminium foil. It was then shaken vigorously. (k)-testing pen placed into the chamber of the ATP bio luminometer. (l)- ATP count of the sample is measured and recorded.

As ATP is a commonly occurring substance, the surface of the swab stick used for the test or the aligner surface was selected for pressing the test stick to avoid any contamination. After this, the upper part of the pen was twisted clockwise until it contacted the lower part of the pen. This caused the aluminium foil of the reagent chamber to slit open with the sharp end of the stick, causing the reagent and the sample to mix. The pen was then shaken vigorously at least 5-10 times to better mix the sample with the reagent unt il a foamy mix was visible. Without any further delay, the pen was then placed into the reading chamber of the HY-Lite 2 ATP bio illuminometer, for immediate measurement of the ATP present on the inner surface of clear aligners. The measurements were then recorded and analysed. **(Fig.3)**

All the patients were asked to fill out an oral hygiene maintenance form describing the oral hygiene measures, dietary intake and method of cleaning the aligners performed by them over the 14 days of aligner wear. These oral hygiene habits were then compared between patients wearing different brands of aligners and correlated with the plaque retention on the surfaces of different aligner brands.

Statistical analysis

Data obtained was entered and sorted in Microsoft Excel (v.2013). Statistical analysis was performed using Statistical Package for social sciences (SPSS) software (v.21.0). Descriptive statistics were performed for the parameters assessed in different groups. Intergroup comparison between aligners and archwise comparison between different groups was performed using One-way Analysis of Variance (ANOVA) and Tukey's post hoc test to assess significant differences. Intragroup comparison between upper and lower arch was done using Unpaired t-test/independent samples t-test. All statistical tests were performed at 95% confidence intervals; keeping the p-value of less than 0.05 as statistically significant. Pearson's correlation coefficient was performed to assess whether there is any relationship between different parameters of case history and different types of aligners.

RESULTS

ATP Bioluminescence was used to evaluate the plaque retention on four leading brands of clear aligners available in the Indian market, labelled as Aligner Brand 1, Aligner Brand 2, Aligner Brand 3 and Aligner Brand 4 for this study. Plaque retention on the aligner surface was evaluated after 14 days of aligner wear, wherein the microbial count on the aligner surface was assessed by measuring the levels of ATP in terms of RLU. The plaque retention on aligners when compared between different groups using the One-way Analysis of Variance (ANOVA) test, showed a statistically insignificant difference between the four groups, (p value >0.05). However, Aligner Brand 2 showed a trend towards higher values of plaque retention followed by Aligner Brand 1 and then Aligner Brand 3, while the Aligner Brand 4 showed the lowest values (Table 1).

Table 1: Mean data of microbial count (plaque retention) in different brands of clear aligners, i.e., Aligner Brand 1, Aligner Brand 2, Aligner Brand 3 and Aligner Brand 4.

Groups	No. of Aligners	Mean (RLU)	Std. Deviation
Aligner Brand 1	26	**73500.00**	15718.51
Aligner Brand 2	26	**76000.00**	18481.27
Aligner Brand 3	26	**71692.30**	17139.14
Aligner Brand 4	26	**71423.07**	16803.75

On pairwise comparison between the four groups, no statistically significant results were obtained (p-value > 0.05). Thus suggesting that the plaque retention in all the four groups was comparable to each other. (Table 2)

Table 2 : Intergroup comparison between the four groups of clear aligners, i.e., Aligner Brand 1,Aligner Brand 2 , Aligner Brand 3, and Aligner Brand 4. The mean differences between the groups showing statistically insignificant results as p-value > 0.05. (I)- Group 1 comprising of 1 brand for comparison, (J) – Group 2 comprising of the other 3 Brands of Aligners for pairwise comparison.

(I) Groups	(J) Groups	Mean Difference (I-J) (RLU)	P value
Aligner Brand 1	Aligner Brand 2	2500	0.945
	Aligner Brand 3	1807.7	0.159
	Aligner Brand 4	2096.93	0.629
Aligner Brand 2	Aligner Brand 1	2500	0.945
	Aligner Brand 3	4307.7	0.413
	Aligner Brand 4	4576.93	0.918
Aligner Brand 3	Aligner Brand 1	1807.7	0.159
	Aligner Brand 2	4307.7	0.413
	Aligner Brand 4	269.23	0.801
Aligner Brand 4	Aligner Brand 1	2096.93	0.629
	Aligner Brand 2	4576.93	0.918
	Aligner Brand 3	269.23	0.801

*- p value <0.05 (statistically significant)

Since plaque retention can be highly varied in different individuals, due to several factors such as oral hygiene habits, dietary intake, or methods of cleaning the appliance, all the patients were given the same instructions on how to maintain their oral hygiene and clean their aligners. On the day of testing the aligners for plaque retention, each patient was asked to fill out an oral hygiene maintenance form regarding the oral hygiene habits they followed in the 14 days of aligner wear. Pearsons correlation coefficient was performed to assess whether there is any relationship between different parameters of oral hygiene maintenance and plaque retention on different types of aligners. A positive correlation was found between the brushing frequency, frequency of mouthwash, and frequency of sugar added drinks consumed with the plaque retention on different brands of clear aligners. (Table 3-Table 7)

Table 3- Correlation between Brushing practice and plaque retention on different aligners.

Parameter		Aligner Brand 1	Aligner Brand 2	Aligner Brand 3	Aligner Brand 4
Brushing in a day	Pearson Correlation (r value)	-.786	-.689	.274	-.171
	p value	.001*	.009*	.364	.576
	N	13	13	13	13

*- p value <0.05 (statistically significant).

Table 4- Correlation between mouthwash practice and plaque retention on different aligners.

Parameter		Aligner Brand 1	Aligner Brand 2	Aligner Brand 3	Aligner Brand 4
Mouthwash in a day	Pearson Correlation (r value)	.567	.382	-.103	.537
	p value	.043*	.198	.737	.051*
	N	13	13	13	13

*- p value <0.05 (statistically significant)

Table 5: Correlation between consumption of Sugar added drinks and plaque retention on different aligners.

Parameter		Aligner Brand 1	Aligner Brand 2	Aligner Brand 3	Aligner Brand 4
Sugar added drinks	Pearson Correlation (r value)	.034	.594	-.307	.229
	p value	.913	.032*	.308	.453
	N	13	13	13	13

*- p value <0.05 (statistically significant).

Table 6- Correlation between consumption of sweets and plaque retention on different aligners.

Parameter		Aligner Brand 1	Aligner Brand 2	Aligner Brand 3	Aligner Brand 4
Sweets consumed	Pearson Correlation (r value)	-.433	.101	-.028*	.024
	p value	.139	.742	.927	.939
	N	13	13	13	13

*- p value <0.05 (statistically significant)

Table 7- Correlation between method of cleaning the aligner and plaque retention on different aligners

Parameter		Aligner Brand 1	Aligner Brand 2	Aligner Brand 3	Aligner Brand 4
Method of cleaning	Pearson Correlation (r value)	-.257	.069	-.349	-.436
	p value	.396	.823	.243	.136
	N	13	13	13	13

*- p value <0.05 (statistically significant)

DISCUSSION

The increased demand for more aesthetic and comfortable orthodontic treatment has fuelled an exponential growth in the clear aligners industry, making it the focal point of most new research and development in the field. Clear aligners offer an edge over the conventional bracket system as they are removable, offering better aesthetics and hygiene control, customized according to patient-specific malocclusions and capable of progressively guiding the teeth into their programmed positions [1,2]. However, not all aligners are created equal, and those currently available in the market vary in terms of their construction material, thickness and clinical protocol. Various studies have evaluated and compared the mechanical properties of commercially available thermoplastic materials and concluded that the thermoplastic materials available in the market have very different mechanical characteristics [14-17]. In terms of oral hygiene, different studies have compared the periodontal health and changes in oral microbial flora with use of clear aligners and conventional brackets.[18-21] Even though most studies suggested that clear aligners, being removable appliances had an obvious advantage over the bracket systems, even with the use of clear aligners studies have shown the incidences of new white spot lesions, thus raising questions on the plaque retention and biofilm adhesion of thermoplastic materials [4,22]. While previous studies have assessed the changes in oral microbial flora with treatment with clear aligners, minimal data exists on comparison of plaque retention between commercially available different clear aligners.

In this study plaque retention on different commercially available clear aligners available in the Indian market was evaluated and compared. The plaque retention was assessed on aligners that had been worn by the patients for 14 days. The results obtained showed a very minimal difference between the four groups of aligners which were of no statistical significance (p-value > 0.05). The results however showed a trend of a slightly higher amount of plaque retention with Aligner Brand 2, followed by Aligner Brand 1, Aligner Brand 3 and the lowest with Aligner Brand 4. This could be due to various factors such as patient variability, the difference in methods of maintaining oral hygiene, different methods of cleaning the aligners or variability in the dietary intake of various patients. As previously reported by Zee *et(remove dot) al*. (1996,1997) the proportions of bacterial species were seen to be significantly varied between rapid and slow plaque formers [23,24]. Similarly, Haffajee *et(remove dot) al.* (2009) found a wide range in total number of organisms in their participants. They also mentioned that when looking at changes in the oral biofilm, factors including nutrition, oral hygiene behaviours, and genetic backgrounds are typically overlooked [25]. Even though patients in this study were instructed via an educational video on how to maintain their oral hygiene, variability was yet noticed. The questionnaire form collected from each patient can be used to see the variability in patient habits and justify the results obtained as a higher frequency of patients brushing thrice daily could be seen with Aligner Brand 4 and Aligner Brand 3 as compared to Aligner Brand 1and Aligner Brand 2. The pearsons correlation coefficient showed a statistically significant relation between frequency of brushing and mouthwash with plaque retention on the aligner surface. The frequency of sugar-added drinks and sweets consumed was seen to be the highest with the Aligner Brand 1, thus justifying its high values of plaque retention. The frequency of consumption of sugar added drinks also showed a statistically significant correlation with the amount of plaque retention on the aligners. Various case reports have reported a higher incidence of enamel demineralization and poor periodontal health with aligner wear in patients that had excessive sugar added drinks while wearing the aligners [4].The method of cleaning the aligner was also seen to be variable in spite of the instructions given to the patients at the beginning of the 14 days of aligner wear. While most patients used a soft toothbrush and paste to clean their aligners, the incidence of patients using only water to rinse their aligners was highest with the Aligner Brand 1group. On the contrary, patients using water and soap to clean their aligners was seen to be the highest with Aligner Brand 3 and Aligner Brand 4. This could be as instructions of the orthodontists performing the treatment had a stronger impact on the patients. Moreover, irregular surfaces of the clear aligners (remove) provide niches and reservoirs for bacterial species, thus promoting the regrowth of existing microbiota [26]. Another factor to be taken into consideration that could lead to the variability in results is the higher number of composite attachments and irregular surfaces on the teeth. The efficiency and amount of interproximal stripping done are factors that can influence plaque adhesion at these sites. Composite attachment and areas with an increased amount of stripping lead to more irregular and rough surfaces that would harbour more micro-organisms and thus, result in a greater amount of plaque retention.

Various studies have compared commercially available thermoplastic materials and clear aligners [14-17]. Lombardo *et(remove dot) al.* (2015) compared the optical properties of 3 different commercially available clear aligners using spectrophotometry and found a significant difference between the three aligners with F22 aligners being the most transparent [15]. The results of this study were similar to those of Turkoz *et(remove dot) al.* (2020) who compared the adherent oral biofilm microorganisms on 4 different commercially available thermoplastic materials and concluded that no significant difference was seen between the 4 groups [27]. However, the thermoplastic material studied was not thermoformed and was studied in artificial saliva. Tamburrino *et(remove dot) al.* (2020) has shown that thermoforming the material has an impact on the mechanical properties of thermoplastic material such as its elastic modulus and yield strength, which could

hamper the adhesion of oral microflora on the material [16]. Also, with no niches and reservoirs, as present in the clear aligners, retention of biofilm on the aligner surface cannot be appropriately assessed.

Thus, the results of this study suggest that while variability in patient oral hygiene habits and dietary intake can influence the plaque retention on clear aligners, different brands of commercially available aligners do not show any significant difference in terms of plaque retention.

DECLARATIONS

- **Ethical approval: Ethical approval by Institutional Ethical Committee** (ECR/328/Inst/MH/2019)
- **Consent for publication: Not Applicable**
- **Data Availability statement: Not applicable**
- **No competing interest**
- **No funding sources**

REFERENCES

1. **Weir T(2017):** Clear aligners in orthodontic treatment. Aust. Dent. J., 62(1): 58-62.
2. **Karkhanechi M, Chow D, Sipkin J *et al.* (2013):** Periodontal status of adult patients treated with fixed buccal appliances and removable aligners over one year of active orthodontic therapy. Angle Orthod., 83(1): 146-151.
3. **Lombardo L, Palone M, Scapoli L, Siciliani G, Carinci F (2021):** Short-term variation in the subgingival microbiota in two groups of patients treated with clear aligners and vestibular fixed appliances: A longitudinal study. Orthod. Craniofac. Res.,24(2): 251-260
4. **Moshiri M, Eckhart J, McShane P, German D(2013):** Consequences of poor oral hygiene during clear aligner therapy. J. Clin. Orthod., 47(8): 494-498.
5. **Carranza F, Newman M, Takei H, Klokkevold P(2012):** Carranza's clinical periodontology. Ed 10. St. Louis, Mo: Elsevier Saunders;. Chapter 9: Microbiology of Periodontal Disease, pp- 306-335.
6. **Buschang P, Chastain D, Keylor C *et al.* (2019):** Incidence white spot lesions among patients treated with clear aligners and traditional braces. Angle Orthod., 89(3): 359-364.
7. **Azeem M, Hamid W(2017):** Incidence of white spot lesions during orthodontic clear aligner therapy. J. World Fed. Orthod., 6: 127-130.
8. **Stanley P (1989):** A review of bioluminescent ATP techniques in rapid microbiology. J. biolumin chemilumin., 4(1): 375-380.
9. **Pellegrini P, Sauerwein R, Finlayson T *et al.* (2009):** Plaque retention by self-ligating vs elastomeric orthodontic brackets: quantitative comparison of oral bacteria and detection with adenosine triphosphate-driven bioluminescence. Am. J. Orthod. Dentofac. Orthop.,135(4): 426-427.
10. **Buck T, Pellegrini P, Sauerwein R, Leo M, Covell A, Maier T, Machida A (2011):** Elastomeric-ligated vs self-ligating appliances: A pilot study examining microbial colonization and white spot lesion formation after 1 year of orthodontic treatment. Orthodontics (Chic),12(2): 108-121.
11. **Elalem M, Abu-Shamaah N, Nadim M(2013):** ATP Bioluminescence: A clinical tool to measure plaque retention on tooth surface around orthodontic brackets J. AM. Sci., 9(7): 335-339.
12. **Baba Y, Sato Y, Owada G, Minakuchi S(2018):** Effectiveness of a combination denture-cleaning method versus a mechanical method: comparison of denture cleanliness, patient satisfaction, and oral health-related quality of life. J. Prosthod. Res., 62(3): 353-358.
13. **Eed H, Abdel-Kader N, El Tahan M, Dai T, Amin R(2016):** Bioluminescence-Sensing Assay for Microbial Growth Recognition. J. Sens., 1:5.
14. **Kohda N, Iijima M, Muguruma T(2013):** Effects of mechanical properties of thermoplastic materials on the initial force of thermoplastic appliances. Angle Orthod., 83(3): 476–483.
15. **Lombardo L, Martines E, Mazzanti V(2017):** Stress relaxation properties of four orthodontic aligner materials: *A 24-hour in vitro study*. *Angle Orthod*., 87(1): 11–18.
16. **Tamburrino F, D'Antò V, Bucci R, Alessandri-Bonetti G(2020):** Mechanical Properties of Thermoplastic Polymers for Aligner Manufacturing: In Vitro Study. J. Dent., 8(2): 47-53.
17. **Daniele V, Macera L, Taglieri G *et al.* (2020):** Thermoplastic disks used for commercial orthodontic aligners: Complete physicochemical and mechanical characterization. Materials., 13(10): 1-10.
18. **Madariaga A, Bucci R *et al.* (2020):** Impact of Fixed Orthodontic Appliance and Clear Aligners on the Periodontal Health: A Prospective Clinical Study. Dent. J., 8(1): 4-11.
19. **Chibber A, Agarwal S, Yadav S, Kuo C, Upadhyay M(2018):** Which orthodontic appliance is best for oral hygiene? A randomized clinical trial. Am. J. Orthod. Dentofac. Orthop.,153(2): 175–183.
20. **Lucchese A, Bonini C, Noviello M *et al.* (2021):** The Effect of removable orthodontic appliances on oral microbiota: A systematic review. Appl. Sci.,11(6): 2881-2889.
21. **Rossini G, Parrini S, Castroflorio T(2015):** Efficacy of clear aligners in controlling orthodontic tooth movement: a systematic review. Angle Orthod.,85(5): 881-889.
22. **Albhaisi Z, Al-Khateeb S, Abu Alhaija E(2020):** Enamel demineralization during clear aligner orthodontic treatment compared with fixed appliance therapy, evaluated with quantitative light-induced fluorescence: A randomized clinical trial. Am. J. Orthod. Dentofac. Orthop.,157(5): 594-601.
23. **Zee K, Samaranayake L, Attström R(1996):** Predominant cultivable supragingival plaque in Chinese 'rapid' and 'slow' plaque formers. J. Clin. Periodontol.,23(11): 1025–103.
24. **Zee K, Samaranayake L, Attström R(1997):** Scanning electron microscopy of microbial colonization of 'rapid' and 'slow' dental-plaque formers in vivo. Arch . Oral Biol., 42(10): 735–742.
25. **Haffajee A, Teles R, Patel M(2009):** Factors affecting human supragingival biofilm composition. II. Tooth position. J. Periodontal Res.,44(4):520-528.
26. **Low B, Lee W, Seneviratne C *et al.* (2011):** Ultrastructure and morphology of biofilms on thermoplastic orthodontic appliances in 'fast' and 'slow' plaque formers. Eur. J. Orthod.,33(5): 577–583.
27. **Türköz C, Canigür Bavbek N, Kale Varlik S *et al.* (2012):** Influence of thermoplastic retainers on Streptococcus mutans and Lactobacillus adhesion. Am. J. Orthod. Dentofac. Orthop.,141(5): 598–603.