AN INVESTIGATION ON THE ORAL MICROBIOTA IN PLAQUE SAMPLES DURING ORTHODONTIC TREATMENT

Abstract

Malocclusion can be corrected by fixed orthodontic appliance therapy. However, the complex appliance design and the material surface characteristics of the appliance make mechanical debriding difficult, enabling biofilm formation**.** This study was performed to investigate the microbial load in plaque at different time periods of the orthodontic therapy in participants with professionally well-maintained oral hygiene. This prospective study was conducted among 12 adult male participants. Six control (C batch) and six under orthodontic treatment (T batches). Simplified oral hygiene index OHI (S), Plaque index (PlI), and Russell's Periodontal index (PI) scoring was performed for all subjects prior to sample collection. Plaque samples were collected from batch T, before the initiation of treatment (T-0), and at various intervals during treatment, 1st week (T-1), 3rd month (T-2), 11th month (T-3), 18th month (T-4) and 3months after the debonding (T-5) i.e. post-treatment period, corresponding with the sequential archwires in regular use. Samples were collected from the C group also in the same time intervals. Six bacterial phyla, *viz*. *Firmicutes, Proteobacteria*, *Bacteroidetes, Actinobacteria*, *Fusobacteria, Spirochaetes* , and one fungal phylum i.e. *Ascomycota* were examined by subculture. Index scoring revealed that hygiene was maintained throughout the study in both the control and treatment batches. A general increase in the microorganisms was noticed, in the treatment group reaching a peak at 18th month of treatment at T4 and reduced to pretreatment levels on debonding. Bacterial and fungal phyla gradually increased, attaining the peak in the T-4 group, followed by a reduction after debonding. Though the microorganisms count increased during treatment, it was not exponential and can be considered favorable flora which matched with the good clinical oral health.

Keywords

Orthodontic treatment, stainless steel brackets, stainless steel wires, Niti wires, plaque, microbial load.

Comment [WRH1]: 1. Please write a situation analysis in the abstract to confirm why the researcher took the topic **2.**Please write down the sampling technique for this research, whether totality sampling or non-probability sampling or whether you want to have probability sampling. what do you mean by totality sampling? Please clarify

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Introduction:

Fixed orthodontic appliance is a method proven for a competent correction of malocclusion.[1] However, the appliances make the oral cavity vulnerable to plaque formation resulting in gingival hyperplasia and inflammation leading to demineralization and white spot lesions.[2] The oral cavity differs from other body surfaces functionally. The position and biological characteristics act as a deciding factor on the existence of oral microbes. The properties are dynamically subjected to regular changes throughout the lifespan of an individual.[3] The human oral microbiome is complex in nature and has been reported to host over 700 bacterial species and 100 fungal species. A majority of them are difficult to grow in the laboratory and hence remain unidentified. Information is provided by a Human Oral Microbiome Database (HOMD) which reveals that there are 687 species belonging to 185 genera and 12 phyla.[4] Increased oral colonization of fungal pathogens, especially *Candida albicans*, in individuals wearing oral appliances was well documented.[5] Many oral factors including placement of fixed orthodontic appliances can cause buccal lesions due to candida growth. Candida is one of the major oral fungal pathogens causing superficial mucosal diseases to deep-rooted mycoses.

Microorganisms exert a unique response against the oral surface in a specific manner involving receptors and adhesion molecules in favor of colonization employing a “lock and key” mechanism.[6] Additional causative factors influencing biofilm formation on teeth and brackets are surface free energy, surface roughness, wettability impact, surface tension, formation of salivary protein layer, etc.[7] Many investigations conducted in the recent past have clearly demonstrated the influence of orthodontic appliances in enhancing biofilm formation irrespective of the nature and design of the wide range of materials used. Luccesse et al.[8] have pointed lacunae that most of the studies failed to distinguish whether the reported microbiota is from the surfaces of appliances or oral mucosa. Attempts have also been made to explore the complex environment favoring the growth of several varieties of microorganisms through metagenomic studies.[9]

Considering a huge diversity observed in the normal oral microflora, the profile when combined with exposure to the different metallic appliances as in Orthodontics has been attributed thus far.[2] , to poor oral hygiene. The present study is an attempt to investigate common microbial populations from plaque samples in orthodontic patients with professionally well-maintained oral health.

Materials and Methods

This prospective study was approved by the Institutional Human Ethics Committee, Saveetha Medical College Hospital, Chennai, India (013/12/2015/IEC/SU). The study was carried out between Jan 2017 and Dec 2018, before COVID 19 pandemic. The study was conducted with twelve male participants, six in the control (C) batch and six in the treatment batch (T) (18 – 45 years of age) who visited the outpatient department of Orthodontics – Saveetha Dental College Hospital, with a complaint of malocclusion. The inclusion criteria were healthy adults with bimaxillary dentoalveolar proclination malocclusion, with a full complement of teeth erupted in the oral cavity, and zero decay / missing / filled teeth index (DMFT) were included. It was also ensured that the participants were not under antibiotics or antiseptic mouthwash for two months prior to the sample collection. The sample size was found to be adequate, assuming 50% improvement in treatment, 20% standard deviation, 90% power, and 5% significance level. After explanations of planned procedures and the study risk / benefits, the queries of participants were answered , and written informed consent for participation in the study was obtained. Gemini MBT 0.022 slot brackets - 3M company were bonded in all cases. The plaque samples were collected from the participants at various intervals referred to as groups C-0 to C-5 and T-0 toT-5. Before initiation of treatment (T-0), during the course of treatment at 1st week after bonding with 0.014 Niti wire (T-1), during 3rd month on 0.016 X 0.022 Niti wire (T-2), during 11th month on 0.019 X 0.025 SS wire (T-3), during 18th month on 0.019 X 0.025 SS with retraction mechanics of coil springs and Class II elastics (T-4) and finally 3 months after debonding (T-5) post-treatment. Simultaneously, the samples were collected from the control group C0 - C5. C batch consisted of adults with the same inclusion criteria who had differed correction for a later period.

All participants underwent periodontal assessment with simplified oral hygiene index OHI(S), Plaque index PII and Russell's PI[10, 11, 12] Scoring prior to sample collection, and a periodic scaling on every appointment after collecting samples. Participants were asked to report for sample collection, without brushing for 24 hrs prior to and before consuming breakfast. The plaque samples were collected individually in a 2 ml disposable sterile vial containing 1 ml normal saline (0.6 M) using a sterile dental probe, from buccal surfaces of the maxillary right and mandibular left first and second permanent molars, from the tooth surface cervical to buccal tubes.

Isolation and Identification of microbial community

The plaque samples were manually agitated to make a uniform suspension. Initially, serialdilutions of plaque samples were made to quantify microorganisms . 1:10, 1:100, 1:1000, 1:10000, 1:100000 dilutions, enough to count on a Petri plate. Following this, 10 µl of the sample was pipetted aseptically and spread uniformly on the entire dish with suitable growth media, including blood agar, MacConkey agar, brain heart infusion agar, and Sabourauds dextrose agar (HiMedia) depending on the type of microorganisms to be investigated. All individual colonies were subcultured using nonselective growth media to get pure cultures. After the incubation period, the developed pure colonies were morphologically examined, stained, and identified by standard microscopic protocols and biochemical methods using HiMedia Identification Kits.

Statistical analysis

Student's t-test for Mean of OHI(S), PII, and Russell's PI scores between the control and treatment batches, was done to assess the oral hygiene status of the participants.

The microbiological data in CFU from plaque samples were entered in Microsoft Excel 2019 version and further analyzed using Sigma Plot 14.5 version (Systat Software Inc, USA). The descriptive statistics for individual microorganisms and significance of testing were done using Oneway Anova for differences in mean values among the control and treatment batches. The multiple comparisons within the group were carried out by Dunnett's Method and between groups by Student's t-test and graphically represented in figures.

Results

Table 1 revealed that both the Simplified Oral Hygiene Index OHI (S) and plaque index PlI scores calculated from C0 – C5 and T0 - T5 during the course of the treatment were good, while

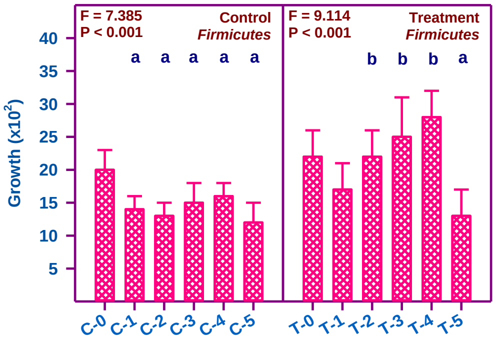
Russell's periodontal index PI scores calculated for C-0 to C-5 and T-0 to T-5 was suggestive of simple gingivitis. No significant difference was observed between batches in OHI(S) (P = 0.591), whereas significant differences were observed in PII and PI (*P* = 0.003 and 0.005, respectively). Although the values were statistically significant, the values were well within clinical limits of simple gingivitis. Overall, the indices revealed well-maintained oral hygiene in control and treatment batches throughout the period of observation.

Table 1: Significance with Student's t-test for Mean scores of OHI(S), PlI and Russell's PI

S. No.	**OHI (S)**		**PII**		**PI**	
	Control	**Treatment**	**Control**	**Treatment**	**Control**	**Treatment**
1	0.68	0.76	0.51	0.60	0.2	0.43
2	0.68	0.61	0.54	0.73	0.4	0.50
3	0.72	0.75	0.5	0.60	0.4	0.73
4	0.72	0.80	0.62	0.73	0.3	0.62
5	0.82	0.81	0.6	0.72	0.5	0.71
6	0.73	0.74	0.5	0.68	0.4	0.60
Mean	0.725	0.745	0.545	0.677	0.367	0.598
S.E.M.	0.021	0.029	0.022	0.025	0.042	0.048
Student's t-test	t = 0.555		t = 3.953		t = 3.639	
	P = 0.591		*P* = 0.003		*P* = 0.005	

An overall comparison of *Firmicutes* load in both the C and T batches is presented in Fig. 1. In the case of C batch, there was a significant difference (F = 7.385; *P* < 0.001) among the control groups. Furthermore, the group-wise comparison made within the control batch i.e. C-1, C-2, C-3, C-4 and C-5 were significantly different from C-0 group. Similarly, a significant variation (F = 9.114; *P* < 0.001) was also noticed in the treatment batch. Contrary, to the C batch, in T batch only T-5 showed significant change as compared to T-0. Meanwhile, T-2, T-3 and T-4 were found significant as compared with C-2, C-3 and C-4, respectively. However, T-1 and T-5 groups did not exhibit any change as compared to C-1 and C-5, respectively.

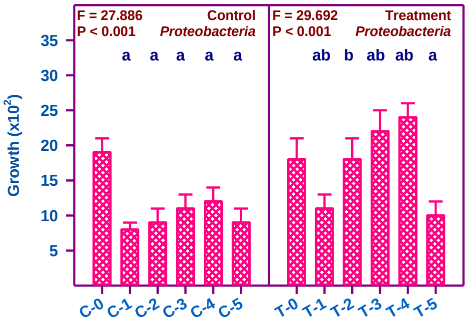
Fig. 1: Comparison of *Firmicutes* load between the control and treatment groups



Values are mean ± SD (n = 6 each); The 'F' and '*P*' values are by one-way ANOVA; [a]Significantly different from C-0/ T-0 batch (Dunnett's test); [b]Significantly different between groups (Student's t- test).

The overall comparison of *Proteobacteria* load made between the control and treatment batches is presented in Fig. 2. In the case of control batch, there was an overall significant difference (F = 27.886; *P* > 0.001) among the control groups. Furthermore, the group-wise comparison was also made and all the control groups i.e. C-1, C-2, C-3, C-4 and C-5 were significantly different from C-0 group. Similarly, a significant variation (F = 29.692; *P* > 0.001) was noticed in the treatment batch. In the case of treatment batch, while T-1 and T-5 groups were found decreased, T-3 and T-4 groups were noticed in high levels as compared to T-0. However, T-1, T-2, T-3 and T-4 groups were significant as compared to their corresponding control groups i.e. C-1, C-2, C-3 and C-4, respectively, while T-5 group did not exhibit any change as compared to C5.

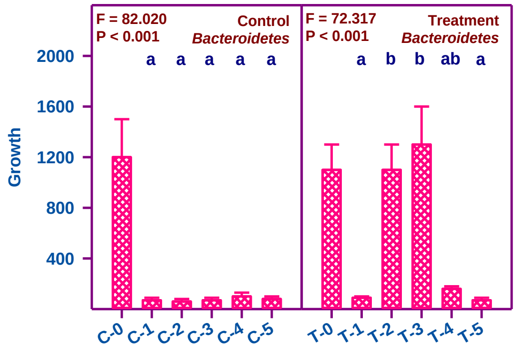
Fig. 2: Comparison of *Proteobacteria* load between the control and treatment groups



Values are mean ± SD (n = 6 each); The 'F' and '*P*' values are by one-way ANOVA; [a]Significantly different from C-0/ T-0 batch (Dunnett's test); [b]Significantly different between groups (Student's t-test).

The overall comparison made between the control and treatment batches of *Bacteroidetes* is presented in Fig. 3. In the case of the control batch, there was a significant difference (F = 82.020; $P > 0.001$) observed among the control groups. Furthermore, the group-wise comparison was also made and all the control groups i.e. C-1, C-2, C-3, C-4, and C-5 were noticed to be significantly decreased several folds from the C-0 group. An overall significant variation (F = 72.317; $P > 0.001$) was also noticed among the treatment groups. A group-wise comparison of the treatment batch indicated that T-1, T-4, and T-5 groups were found decreased as compared to T-0, while T-2 and T-3 groups did not show any change at all. However, T-2, T-3, and T-4 groups were found to be significantly elevated as compared to their corresponding C-2, C-3, and C-4 groups, respectively.

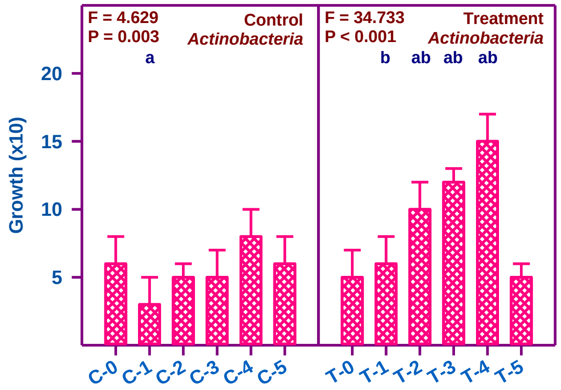
Fig. 3: Comparison of *Bacteroidetes* load between the control and treatment groups



Values are mean ± SD (n = 6 each); The ‘F’ and ‘*P*’ values are by one-way ANOVA; [a]Significantly different from C-0/ T-0 batch (Dunnett’s test); [b]Significantly different between groups (Student’s t- test).

An overall comparison of *Actinobacteria* load in both the control and treatment batches were made and presented in Fig. 4. In the case of control batch, there was a significant difference (F = 4.629; *P* = 0.003) observed among the control groups. Furthermore, the group-wise comparison was also made and only C-1 was significantly lower than C-0 group. Similarly, an overall significant variation (F = 34.733; *P* < 0.001) was also observed in the treatment batch. Only T2, T3 and T4 groups showed significant increase as compared to T-0. Meanwhile, T1, T-2, T-3 and T-4 groups were also found significantly higher as compared to their respective control groups i.e. C1, C-2, C-3 and C-4, respectively, while T-5 group did not exhibit any change when compared to C5.

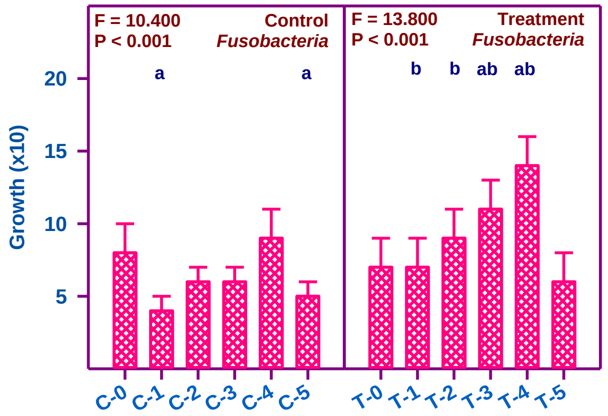
Figure 4: Comparison of *Actinobacteria* load between the control and treatment groups



Values are mean ± SD (n = 6 each); The 'F' and '*P*' values are by one way ANOVA; [a]Significantly different from C-0/ T-0 batch (Dunnett's test); [b]Significantly different between groups (Student's t- test).

The overall comparison of *Fusobacteria* load in both the control and treatment batches is made and the same is presented in Fig. 5. In the case of the control batch, there was an overall significant difference (F = 10.400; *P* < 0.001) observed among the control groups. Furthermore, the group-wise comparison was also made and only C-1 and C-5 groups were found to significantly decrease as compared to the C-0 group. Furthermore, an overall comparison was made in the treatment batch and there was a significant variation (F = 13.800; *P* < 0.001) among the treatment groups. T-2, T-3, and T-4 groups were found significantly elevated as compared to the T-0 group. Meanwhile, T1, T-2, T-3, and T-4 were also found significantly higher as compared to their respective control groups i.e. C1, C-2, C-3, and C-4, while the T-5 group did not exhibit any change when compared to the C-5 group.

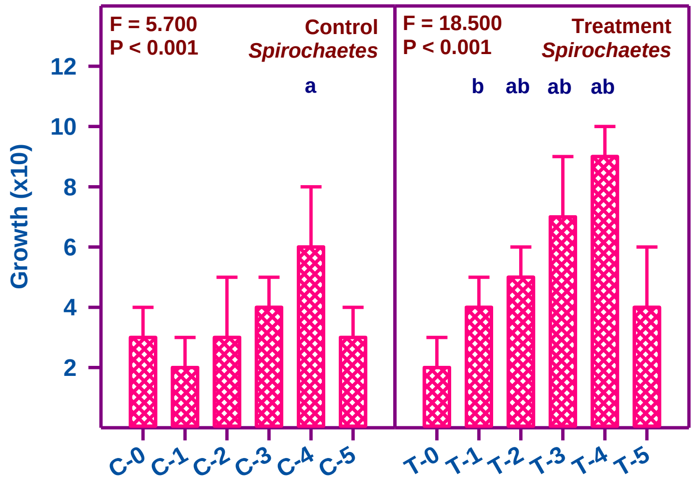
Figure 5: Comparison of *Fusobacteria* load between the control and treatment groups



Values are mean ± SD (n = 6 each); The 'F' and '*P*' values are by one way ANOVA; [a]Significantly different from C-0/ T-0 batch (Dunnett's test); [b]Significantly different between groups (Student's t- test).

The overall comparison of *Spirochaetes* load made in both the control and treatment batches is presented in Fig. 6. In the case of control batch, there was a significant difference (F = 5.700; $P < 0.001$) observed among the control groups. Furthermore, the group-wise comparison was also made within the control batch and only C-4 was found significantly elevated when compared to C-0 group. Similarly, an overall significant (F = 18.500; $P < 0.001$) variation was noticed in the case of the treatment batch. Only T-2, T-3 and T-4 groups were noticed significantly high as compared to T-0. Nevertheless, T1, T-2, T-3 and T-4 groups were observed significantly high as compared to their corresponding control groups i.e. C1, C-2, C-3 and C-4, while T-5 group did not exhibit any change as compared to C5

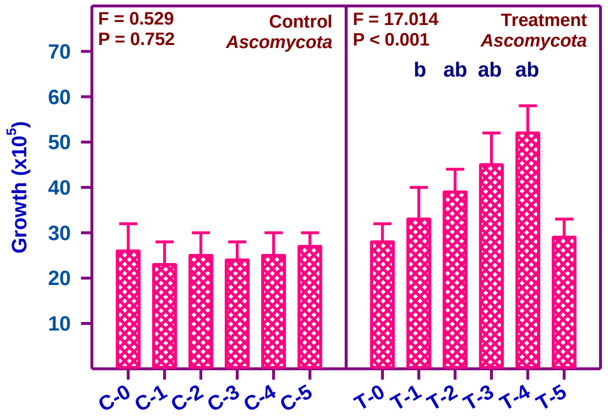
Figure 6: Comparison of *Spirochaetes* load between the control and treatment groups



Values are mean ± SD (n = 6 each); The ‘F’ and ‘*P*’ values are by one-way ANOVA; [a]Significantly different from C-0/ T-0 batch (Dunnett’s test); [b]Significantly different between groups (Student’s t- test).

An overall comparison of *Ascomycota* load in both the control and treatment batches is made and the same is presented in Fig. 7. In the case of control batch, there was no significant difference (F = 0.529; *P =0.752*) observed among the control groups. However, a significant variation (F = 17.014; *P* < 0.001) was noticed in the case of treatment batch. The groups T-2, T-3, and T-4 were seen significantly increased as compared to T-0. Meanwhile T1, T-2, T-3 and T-4 groups were also found significantly high as compared to their corresponding control groups i.e. C1, C-2, C-3, and C-4, respectively. However, the T-5 group did not elicit any change as compared to C5.

Figure 7: Comparison of *Ascomycota* load between the control and treatment groups



Values are mean ± SD (n = 6 each); The 'F' and '*P*' values are by one-way ANOVA; [a]Significantly different from C-0/ T-0 batch (Dunnett's test); [b]Significantly different between groups (Student's t- test).

Discussion:

With mean scores of OHI(S), PlI and Russell's PI well within acceptable clinical limits of simple gingivitis, Table 1 confirms that oral hygiene status was very well maintained among all the participants both in control and in treatment, groups in this study. Literature is replete with studies quite contrary to the present study which probably is the true reflection if adequate professional oral prophylaxis is not given during Orthodontics.

The study evaluated seven phyla, namely, *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Fusobacteria*, *Spirochaetes*, *Ascomycota* from the plaque samples of study participants. In Control batch, there was an overall statistical significant difference within groups among bacterial phyla (Fig 1-6) with marginally reduced counts after C-1. Ascomycota (Fig7), however showed no difference. The result, can probably be attributed to Hawthrone effect among the control participants who carefully maintained oral hygiene.

In Treatment group (Fig 1-7) there was an overall significant difference within and between groups, amongst all organisms studied, particularly at T-2, T-3, T-4 in most organisms. A trend with sequential rise in microbial count was observed from T-2, T-3, to the peak at T-4, at 18 months duration of treatment associated with the stainless steel wires , in retraction mechanics, in all organisms, followed by a reduction at T-5 comparable with C-5 and T-0 in all organisms. This can be inferred as restoration of microbial flora to normalcy after orthodontic treatment. The increase in counts though statistically significant within and between groups was not exponential in any of the organisms in the treatment samples.

The rise at T2 was associated with 16*22 Niti wire ligated with polymeric models during 11 months in treatment, T3 counts were associated with 19*25 stainless-steel archwire with steel ligation, whereas T4 counts were associated with 19*25 stainless-steel archwire with steel ligation during complex retraction mechanics assembly with Niti coil spring, and Class II elastics for retraction during extraction space closure phase of Orthodontic treatment.

The population trend seen in the plaque samples was as; *Ascomycota* (10^5) observed to be highly dominating in terms of population size (10^5) compared with other organisms *Firmicutes*(10^2), *Proteobacteria*(10^2) *Bacteroidetes* , *Actinobacteria(10), Fusobacteria(10), Spirochaetes(10).*

Orthodontic appliances do disturb the normal balance and favor colonization of various complex microbiota.[13] This is because plaque removal becomes difficult to perform with the bonded orthodontic appliance. The results of the present study, also show a minimal rise of microorganisms with the course of orthodontics in spite of the good prophylaxis given. Malgorzata et al[14] assessed the level of plaque in the oral cavity during the orthodontic treatment phase and concluded that there exists a 2 to 3 times higher level of plaque, along the gingival margin and around orthodontic brackets. This is more reliable particularly with the metallic orthodontic appliances, which have been reported to initiate specific changes like decreased pH and increased plaque accumulation in the oral cavity. [15,16] Surface roughness of stainless steel archwires attracts more plaque retention, hence the need for smoother wires.[17] This is due to the critical surface tension and adhesion on stainless steel, making them more prone to increased microbial attachment[18]. This is in accordance with the results of the present study with the observation of peak microbial count during the 18th month of treatment using stainless steel archwires

A systematic review by Guo et al on the microbial changes of subgingival plaque during fixed orthodontic therapy showed that there will be a transient increase in the quantity during the first three months followed by a gradual decrease over several months after the removal of the fixed appliance [19]. In the present study, the composition of plaque microflora differed during various periods of orthodontic therapy. The phylum *Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Fusobacteria,* and *Spirochaetes* were found to be increased during the course of orthodontic therapy. This was in accordance with the study that showed a greater prevalence of *Firmicutes, Proteobacteria,* and *Spirochetes* after one month of orthodontic treatment.[20]

The microbial load of periodontitis-associated bacteria in orthodontic appliances using PCR and various molecular techniques has reported the raised level of Eubacterium belonging to the phylum *Firmicutes, Proteobacteria, Actinibacter, Bacterioidetes* in the orthodontic appliance associated plaque.[21-24] This evidence is further strengthened with the present observation on orthodontic plaque samples. Kim et al evaluated the level of periodontitis-associated pathogens during different periods of orthodontic therapy in different individuals and concluded that the bacteria of the phylum *Proteobacteria* and *Bacteroidetes* were significantly increased even from the early stage of orthodontic therapy.[23] A study by Anhoury et al evidenced the higher mean level of *Treponema denticola, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Streptococcus anginosus,* and *Eubacterium nodatum,* all of which belonged to *Spirochetes, Proteobacteria, Fusobacterium, Firmicutes* [25, 26] Tanner et al. [27], reported the genus *Prevotella,* detected in plaque at high gingivitis group of orthodontic patients. Marcela et al [28] reported the predominance of periodontal pathogens of the orange complex in large numbers in association with orthodontic metallic brackets with the help of checkerboard DNA-DNA hybridization in an invivo study.

Liu et al demonstrated a significantly increased level of *Porphyromonas gingivalis* that belongs to Bacteroidetes in the subgingival plaque during the fixed orthodontic treatment. Though they were found to decrease after debonding, their quantity level might be high for 6 months after removal in certain patients which is in disagreement with the results of the present study.[29]

The changes in the frequency of *P.gingivalis, T.forsythia, P.intermedia* after the placement and removal of the fixed orthodontic appliance using polymerase chain reaction were reported earlier.[30]The result of the present study was similar to the above study, revealing that there exists a nonsignificant change in plaque microflora before and after the removal of fixed appliances which could be due to the existence of better oral hygiene Certain components of fixed orthodontic appliances such as molar bands, brackets and wires facilitate the increased adherence and colonization of microorganisms. This can be noticed in the early phase of the treatment that becomes consistent during the first three months initially by orange complex species and later by red complex species.[31]

In a longitudinal study, *P. gingivalis*, *F. nucleatum*, *C. rectus*, *T. denticola* and *T. forsythia* significantly increased which belonged to the phylum of *Fusobacteria*, *Proteobacteria*, *Spirochetes* respectively.[32] The quality of biofilm with increased microbial load particularly of anaerobes is attributed to the complex structure of brackets making it difficult for oxygen to infiltrate the dental plaque on the orthodontic bracket bonded on the tooth surface. Thus, there exists an anaerobic environment promoting obligate anaerobes including *Firmicutes, Bacteroidetes, Fusobacteria* to colonize and grow. [33-35]

Literature is replete with reports of a significant increase in *Candida* during the treatment phase, that reach pretreatment levels on debonding, [36-39] and confirming the importance of hygiene maintenance in Orthodontics

A study on the impact of the removal of the orthodontic appliance on oral microbial changes concluded that there is a significant reduction in the level of periodontal pathogens after debonding.[40] In our present study, it is observed that the 3 months after debonding the orthodontic appliance the plaque samples returned to pretreatment levels. The reduction in microbial level during the post-treatment period in this present study was similar to these previous study results. Michelle et al,[41] also reported that the level of *Bacteroidetes, Proteobacteria, Fusobacteria*, *Spirochetes* increased significantly after 6 months of fixed appliance treatment, and returned to pretreatment levels by 12 months of orthodontic treatment and concluded that orthodontic appliance does not increase the risk due to oral pathogens.

The present study was unique among other studies reported, in that the microbial counts were high in spite of meticulous oral hygiene maintained visibly and statistically (Table 1), by each of the participants and periodic scaling is done by the operator at every appointment. Also need to mention that in most initial appointments the number of plaque samples available was very less. It is imperative to say that, there was no white spot lesion found in any of the participants during the entire period of study, checked. In spite of the very favorable clinical situation, the organism count increased throughout the duration of treatment. The increase, however, was not exponential, which can be attributed to the hygiene maintained. The increase in microbial load can be attributed to the association of metals, elastomeric, and the complicated design of the appliance. Smooth surface by surface treatment of Stainless-steel wire has been recommended to prevent bio-hostability.[42] Among other wires used in orthodontics, SS is by far the smoothest followed by TMA and Niti. The increase in microbial load in T1, T2 can be attributed to the Niti wires ligated with polymeric modules. Polymeric modules have been reported to host organisms.[43] T3 had SS wire with steel ligations. The organism count yet remained on the increase. It may be necessary to explore the metals leached from the appliance assembly. T4 had SS wire with steel ligations, but the complex design of retraction mechanics using Niti coil spring and Class II elastics probably contributed to the conducive atmosphere for peak rise of the microbial content which needs to be further explored.

The clinical significance could be posted with the present results regarding the use of less bio hostable materials, efficient plaque control measures, or with less complicated appliance designs for patients who have problems with oral hygiene.

Conclusion

The results of the present study revealed that the tested phylum counts were found to increase in the plaque samples sequentially reaching a peak during the 18th month of orthodontic treatment and subsequently reduced on debonding to the level of pretreatment. The increase was not exponential in any organism studied.

Subsequently, the rise was associated with a clinically well-maintained oral hygiene, showing that the quantity and quality of organisms reported in the present study, can be assumed to be favorable as far as the metallic orthodontic appliance is concerned for a period of 18 – 20 months under professional prophylaxis.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References:

1. Jasper JJ, McNamara Jr JA. The correction of interarch malocclusions using a fixed force module. American Journal of Orthodontics and Dentofacial Orthopedics. 1995 Dec 1;108(6):641-50.

2. Saloom HF, Mohammed-Salih HS, Rasheed SF. The influence of different types of fixed orthodontic appliances on the growth and adherence of microorganisms (in vitro study). Journal of clinical and experimental dentistry. 2013 Feb;5(1):e36.

3. Ko-Adams C, Cioffi I, Dufour D, Nainar SH, Lévesque CM, Gong SG. Short-term effects of the fixed orthodontic appliance on concentrations of *mutans streptococci* and persister cells in adolescents. American Journal of Orthodontics and Dentofacial Orthopedics. 2020 Mar 1;157(3):385-91.

4. Chen T, Yu WH, Izard J, Baranova OV, Lakshmanan A, Dewhirst FE. The Human Oral Microbiome Database: a web-accessible resource for investigating oral microbe taxonomic and genomic information. Database. 2010 Jan 1;2010.

5. Budtz-Jörgensen E. Etiology, pathogenesis, therapy, and prophylaxis of oral yeast infections. Acta Odontologica Scandinavica. 1990 Jan 1;48(1):61-9.

6. Chattopadhyay I, Verma M, Panda M. Role of oral microbiome signatures in diagnosis and prognosis of oral cancer. Technology in cancer research & treatment. 2019 Aug 1;18.

7. Dittmer MP, Hellemann CF, Grade S, Heuer W, Stiesch M, Schwestka-Polly R, Demling AP. Comparative three-dimensional analysis of initial biofilm formation on three orthodontic bracket materials. Head & face medicine. 2015 Dec;11(1):1-6.

8. Lucchese A, Bondemark L, Marcolina M, Manuelli M. Changes in oral microbiota due to orthodontic appliances: a systematic review. Journal of Oral Microbiology. 2018 Jan 1;10(1):1476645.

9. Sunagawa S, Mende DR, Zeller G, Izquierdo-Carrasco F, Berger SA, Kultima JR, Coelho LP, Arumugam M, Tap J, Nielsen HB, Rasmussen S. Metagenomic species profiling using universal phylogenetic marker genes. Nature methods. 2013 Dec;10(12):1196-9.

10. Greene JG, Vermillion JR. The simplified oral hygiene index. The Journal of the American Dental Association. 1964 Jan 1;68(1):7-13.

11. Russel AL. The periodontal index. J Periodontol. 1967;38(6):585-591.

12. Löe H. The gingival index, the plaque index, and the retention index systems. The Journal of Periodontology. 1967 Nov;38(6):610-6.

13. Freitas AOA, Marquezan M, Nojima MCG, Alviano,DS, Maia LC. The influence of orthodontic fixed appliances on the oral microbiota: A systematic review. Dental Press J Orthod. 2014 Mar-Apr;19(2):46-55.

14. Klukowska M, Bader A, Erbe C, Bellamy P, White DJ, Anastasia MK, Wehrbein H. Plaque levels of patients with fixed orthodontic appliances measured by digital plaque image analysis. American Journal of Orthodontics and Dentofacial Orthopedics. 2011 May 1;13.

15 . Balenseifen JW, Madonia JV. Study of dental plaque in orthodontic patients. J Dent Res. 1970;49:320–324.

16 Chatterjee R, Kleinberg I. Effect of orthodontic band placement on the chemical composition of human incisor tooth plaque. Arch Oral Biol. 1979;24:97–100.

17 Kielan-Grabowska Z, Bacela J, Ziety A, Seremak W, Gawlik Maj M, Kawala B, Borak B, Detyna J, Sarul M. Improvement of Properties of Stainless Steel Orthodontic Archwire Using TiO2:Ag Coating. Symmetry **2021**, 13, 1734.

18 Eliades T, Eliades G, Brantley WA. Microbial attachment on orthodontic appliances: I. Wettability and early pellicle formation on bracket materials. American Journal of Orthodontics and Dentofacial Orthopedics. 1995 Oct 1;108(4):351-60.

19. Guo R, Lin Y, Zheng Y, Li W. The microbial changes in subgingival plaques of orthodontic patients: a systematic review and meta-analysis of clinical trials. BMC Oral Health. 2017 Dec;17(1):1-0.

20. Mártha K, Lőrinczi L, Bică C, Gyergyay R, Petcu B, Lazăr L. Assessment of periodontopathogens in subgingival biofilm of banded and bonded molars in early phase of fixed orthodontic treatment. Acta microbiologica et immunologica Hungarica. 2016 Mar;63(1):1.

21. Demling A, Demling C, Schwestka-Polly R, Stiesch M, Heuer W. Short-term influence of

lingual orthodontic therapy on microbial parameters and periodontal status. A preliminary study Angle Orthod, 80 (2010), pp. 480-484.

22. Thornberg M.J, Riolo C.S, Bayirli B, Riolo M.L, Van Tubergen E.A, Kulbersh R, Periodontal pathogen levels in adolescents before, during, and after fixed orthodontic appliance therapy Am J Orthod Dentofac Orthop, 135 (2009), pp. 95-98. 23.

23. Kim SH, Choi DS, Jang I, Cha BK, Jost-Brinkmann PG, Song JS. Microbiologic Changes in Subgingival Plaque Before and During the Early Period of Orthodontic Treatment. The Angle orthodontist. 2012 Mar;82(2):254-60.

24. Komori R, Sato T, Takano-Yamamoto T, Takahashi N. Microbial composition of dental plaque microflora on first molars with orthodontic bands and brackets, and the acidogenic potential of these bacteria. Journal of Oral Biosciences. 2012 May 1;54(2):107-12.

25. Anhoury P, Nathanson D, Hughes CV, Socransky S, Feres M, Chou LL. Microbial profile on metallic and ceramic bracket materials. The Angle Orthodontist. 2002 Aug;72(4):338-43.

26. Nadkarni, M. A. et al. Pattern of distribution of Prevotella species/phylotypes associated with healthy gingiva and periodontal disease. Eur. J. Clin. Microbiol. Infect. Dis. 31, 2989–2999.

27. Tanner, A. C. et al. White-spot lesions and gingivitis microbiotas in orthodontic patients. J. Dent. Res. 91, 853–858.

28. Andrucioli MC, Nelson-Filho P, Matsumoto MA, Saraiva MC, Feres M, De Figueiredo LC, Martins LP. Molecular detection of in-vivo microbial contamination of metallic orthodontic brackets by checkerboard DNA-DNA hybridization. American journal of orthodontics and dentofacial orthopedics. 2012 Jan 1;141(1):24-9.

29. Liu H, Sun J, Dong Y, Lu H, Zhou H, Hansen BF, Song X. Periodontal health and relative quantity of subgingival Porphyromonas gingivalis during orthodontic treatment. The Angle Orthodontist. 2011 Jul;81(4):609-15.

30. Živković-Sandić M, Popović B, Čarkić J, Nikolić N, Glišić B. Changes in subgingival

microflora after placement and removal of fixed orthodontic appliances. Srpski arhiv za celokupno lekarstvo. 2014;142(5-6):301-5.

31. Contaldo M, Lucchese A, Lajolo C, Rupe C, Di Stasio D, Romano A, Petruzzi M, Serpico R. The oral microbiota changes in orthodontic patients and effects on oral health: An overview. Journal of Clinical Medicine. 2021 Jan;10(4):780.

32. Marczyńska-Stolarek M, Chomicz L. Examination of oral biofilm microbiota in patients using fixed orthodontic appliances in order to prevent risk factors for health complications. Annals of Agricultural and Environmental Medicine. 2019;26(2):231-5.

33. N. Takahashi, K. Saito, C.F. Schachtele, T. Yamada. Acid tolerance and acid-neutralizing activity of Porphyromonas gingivalis, Prevotella intermedia and Fusobacterium nucleatum Oral Microbiol Immunol, 12 (1997), pp. 323-328.

34. Loesche W.J, Gusberti F, Mettraux G, Higgins T, Syed S. Relationship between oxygen tension and subgingival bacterial flora in untreated human periodontal pockets Infect Immun, 42 (1983), pp. 659-667.

35. Takahashi N. Microbial ecosystem in the oral cavity: Metabolic diversity in an ecological niche and its relationship with oral diseases Int Cong Ser, 1284 (2005), pp. 103-112.

36. Sanz-Orrio-Soler I, de Luxán SA, Sheth CC. Oral colonization by Candida species in orthodontic patients before, during and after treatment with fixed appliances: A prospective controlled trial. Journal of Clinical and Experimental Dentistry. 2020 Nov;12(1.

37. Grzegocka K, Krzyściak P, Hille-Padalis A, Loster JE, Talaga-Ćwiertnia K, Loster BW. Candida prevalence and oral hygiene due to orthodontic therapy with conventional brackets. BMC Oral Health. 2020 Dec;20(1):1-9.

38. Muzurovic S, Babajic E, Masic T, Smajic R, Selmanagic A. The relationship between oral hygiene and oral colonisation with Candida species. Medical Archives. 2012 Nov 1;66(6):415.

39. Klaus K, Eichenauer J, Sprenger R, Ruf S. Oral microbiota carriage in patients with

multibracket appliance in relation to the quality of oral hygiene. Head & face medicine. 2016 Dec;12(1):1-7.

40. Topaloglu-Ak A, Ertugrul F, Eden E, Ates M, Bulut H. Effect of orthodontic appliances on oral microbiota—6 month follow-up. Journal of Clinical Pediatric Dentistry. 2011 Jul 1;35(4):433-6.

41. Sallum EJ, Nouer DF, Klein MI, Gonçalves RB, Machion L, Sallum AW, Sallum EA. Clinical and microbiologic changes after removal of orthodontic appliances. American Journal of Orthodontics and Dentofacial Orthopedics. 2004 Sep 1;126(3):363-6.

42. Yu JH, Wu LC, Hsu JT, Chang YY, Huang HH, Huang HL. Surface roughness and topography of four commonly used types of orthodontic archwire. J Med Biol Eng. 2011 Nov;31(5):367-70.

43. Sawhney R, Sharma R, Sharma K. Microbial Colonization on Elastomeric Ligatures during Orthodontic Therapeutics: An Overview. Turk J Orthod 2018; 31: 21-25.