

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 (PII) and Russell's Periodontal index (PI) scoring was performed for all subjects prior to sample collection. Plaque samples were collected from both 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 3 months after the debonding (T-5) i.e. post treatment period, corresponding with the sequential archwires in regular use. Samples were collected from C group also in the same time intervals.~~ and C at similar multiple intervals, corresponding with the sequential archwires for group T in regular use. Six bacterial phyla ~~viz. —Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Fusobacteria, Spirochaetes~~ and one fungal phyla ~~i.e. Ascomycota~~ were examined by subculture. Index scoring revealed that hygiene was maintained throughout study in both the control and treatment batches. A general increase in the microorganisms was noticed, in treatment group reaching a peak at 18th month of treatment at T4 and reduced to pretreatment levels on debonding. Though, the microorganisms count increased during treatment, it was not exponential and can be considered favourable flora which matched with the good clinical oral health.

Keywords

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

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. **As no study has reported assessment of the clinical hygiene status along with the microbial flora at various stages of orthodontic treatment,** the present study, is an attempt to investigate common microbial populations from plaque samples in orthodontic patients with professionally well maintained oral health **over a complete treatment duration .**

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. **A non-probability purposive sampling technique was used to recruit the participants.** 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 mean CFU/ml of organisms from the pilot study was used to calculate the sample size. On keeping the power of 90% and significance level of 5%, total sample size obtained was 12. Thus 6 samples were recruited in each group. 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 to T-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, serial dilutions 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

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). **Normal distribution of the collected was assessed using Shapiro-Wilk numerical test. It was found that the data was normally distributed. Hence, parametric tests such as One-Way ANOVA and Independent t test were carried out.** The descriptive statistics for individual microorganisms and significance of testing were done using One-way 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. 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. **Control for the confounding variable was controlled by stratification, matching for age and gender. p value <0.05 was considered to be statistically significant.**

Results

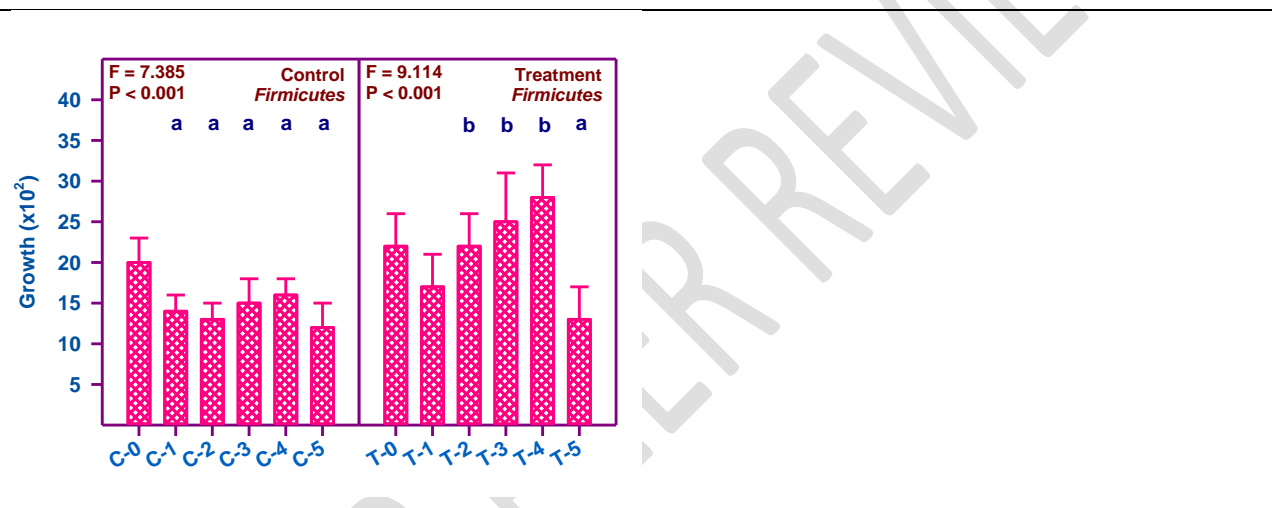
Table 1 revealed that both the Simplified Oral Hygiene Index OHI (S) and plaque index PII scores calculated from C0 – C5 and T0 - T5 during the course of the treatment were good, while Russell's periodontal index PI scores calculated for C-0 to C-5 and T-0 to T-5 was suggestive of simple gingivitis. 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), PII 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

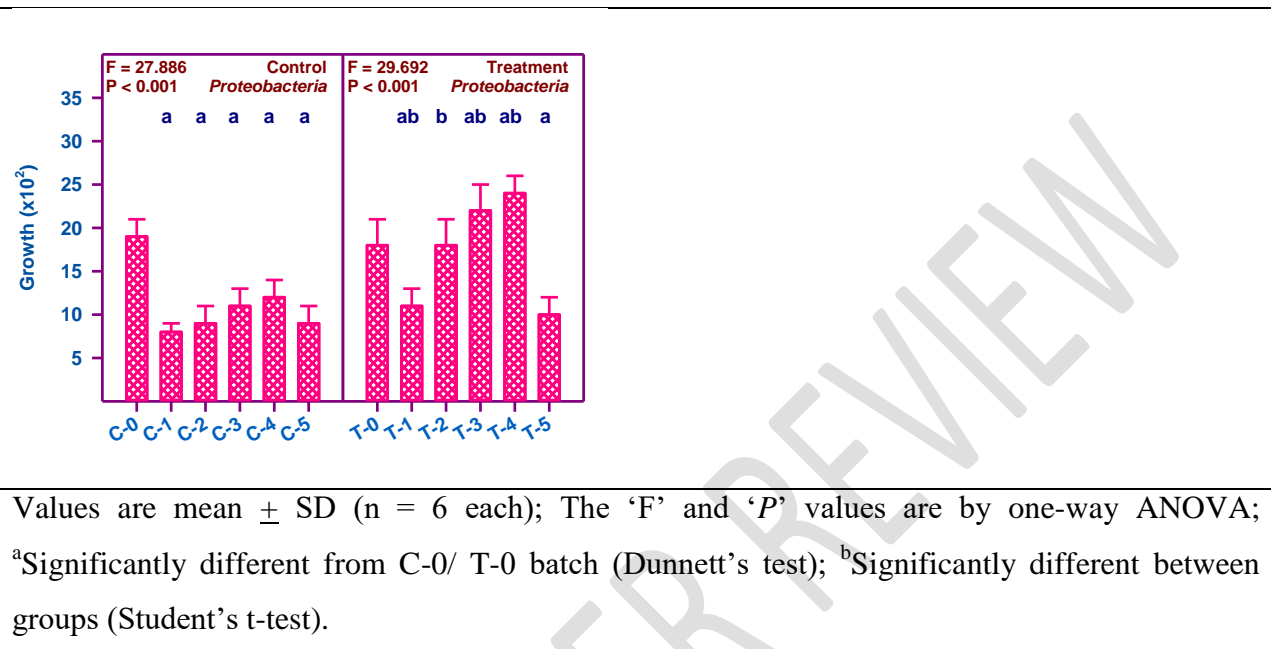


Values are mean \pm SD ($n = 6$ each); The 'F' and 'P' values are by one-way ANOVA; ^aSignificantly different from C-0/ T-0 batch (Dunnett's test); ^bSignificantly different between groups (Student's t- test).

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

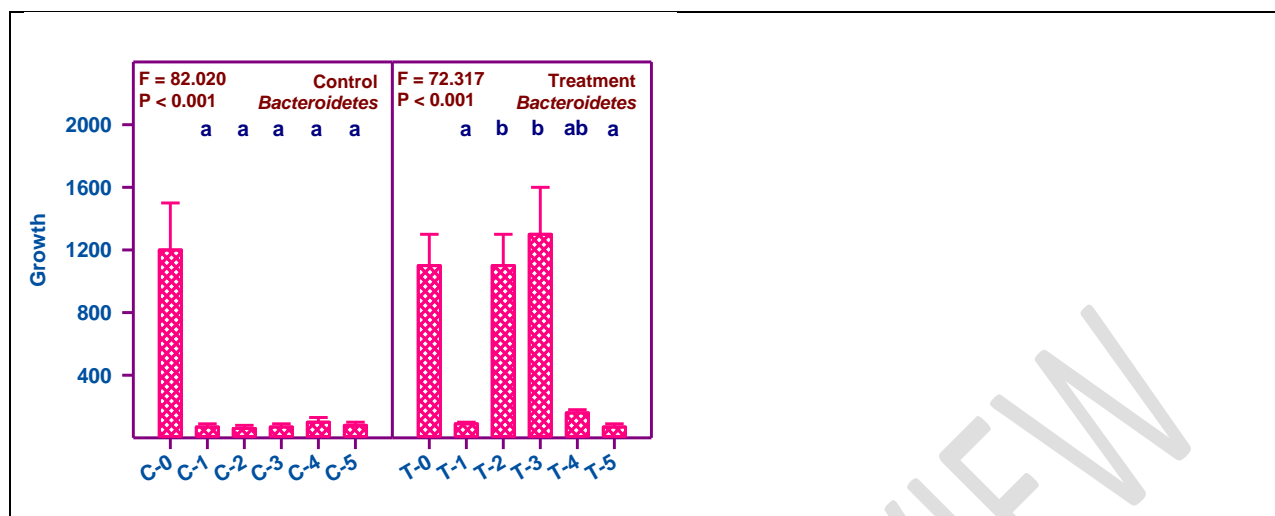
i.e. C-1, C-2, C-3 and C-4, respectively, while T-5 group did not exhibit any change as compared to C5.

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



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.

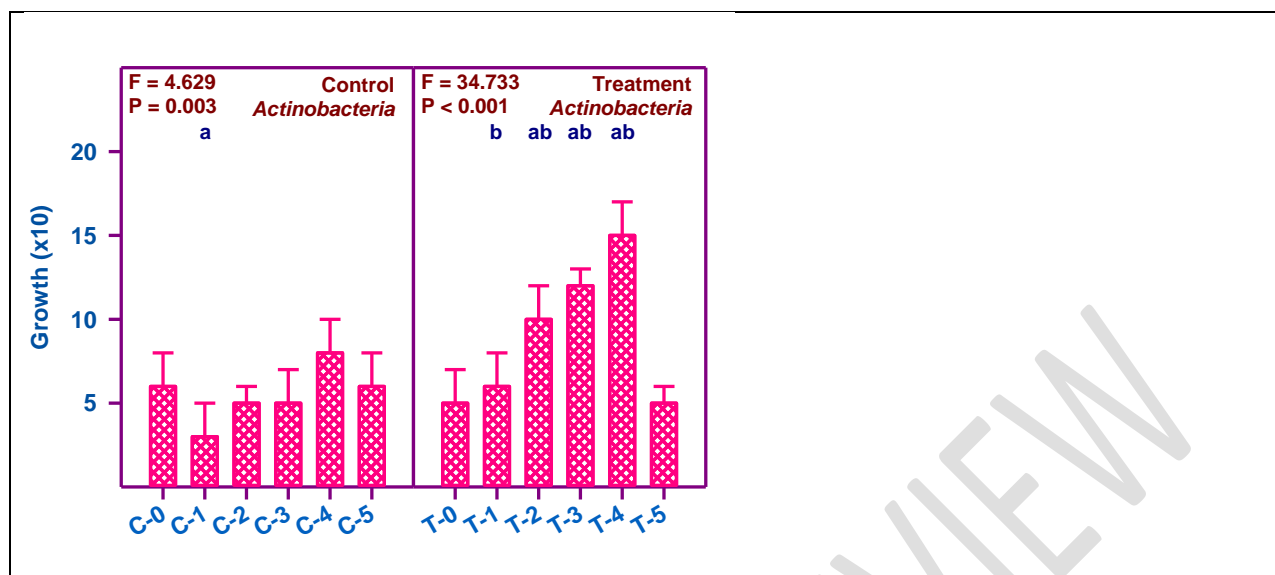
Fig. 3: Comparison of *Bacteroidetes* load between the control and treatment groups



Values are mean \pm SD (n = 6 each); The 'F' and 'P' values are by one-way ANOVA; ^aSignificantly different from C-0/ T-0 batch (Dunnnett's test); ^bSignificantly 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.

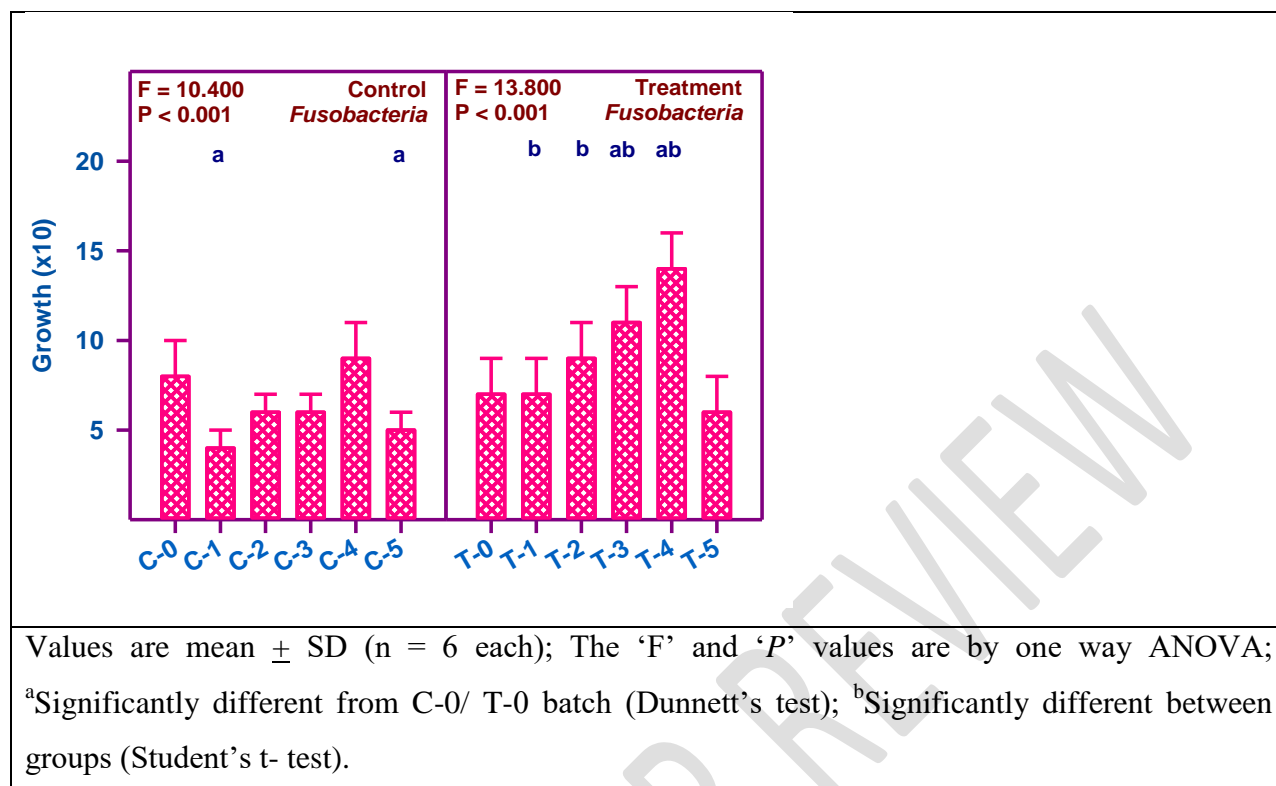
Figure 4: Comparison of *Actinobacteria* load between the control and treatment groups



Values are mean \pm SD (n = 6 each); The 'F' and 'P' values are by one way ANOVA; ^aSignificantly different from C-0/ T-0 batch (Dunnett's test); ^bSignificantly different between groups (Student's t- test).

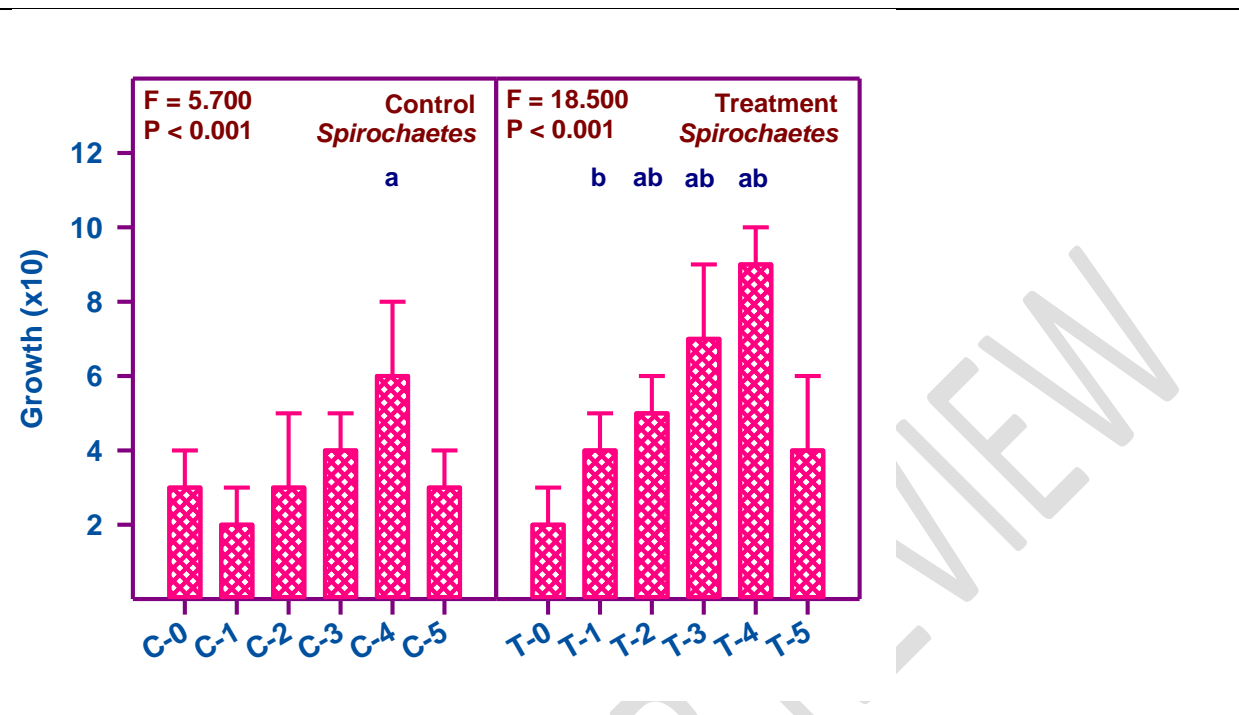
The overall comparison of *Fusobacteria* load in both the control and treatment batches is made and the same is presented in Fig. 5. In the case of the control batch, there was an overall significant difference (F = 10.400; P < 0.001) observed among the control groups. Furthermore, the group-wise comparison 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



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

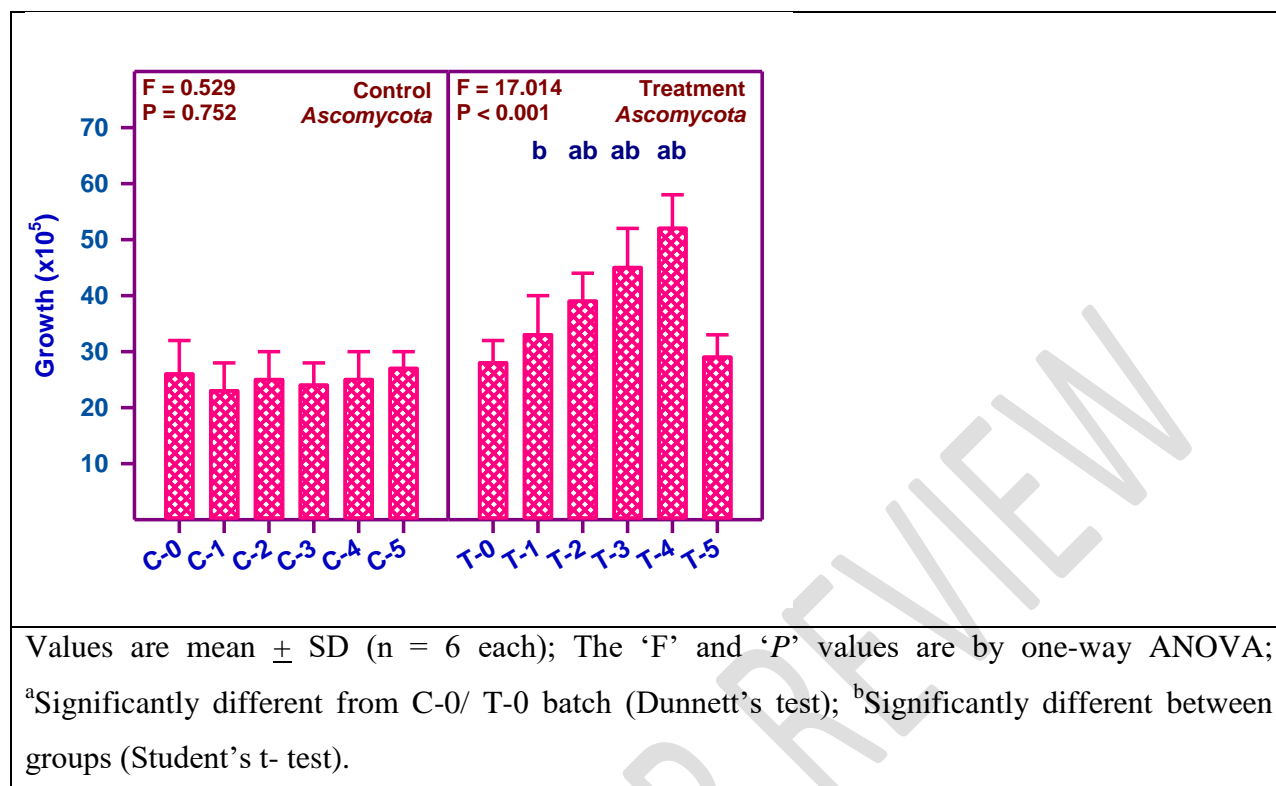
Figure 6: Comparison of *Spirochaetes* load between the control and treatment groups



Values are mean \pm SD (n = 6 each); The ‘F’ and ‘P’ values are by one-way ANOVA; ^aSignificantly different from C-0/ T-0 batch (Dunnett’s test); ^bSignificantly 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



Discussion:

With mean scores of OHI(S), PII 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 Hawthorne 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*, *Actinobacter*, *Bacteroidetes* 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 Sallum EJ](#),^[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. **Further longitudinal studies with more sample size considering the cultural and behavioral variability are needed to extrapolate the results.**

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.

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