South Asian Journal of Research in Microbiology

Volume 18, Issue 8, Page 41-51, 2024; Article no.SAJRM.121137
ISSN: 2582-1989

Orthodontic Appliances and Microbial Dysbiosis: Insights and Implications

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/sajrm/2024/v18i8381

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/121137

Review Article

Received: 10/06/2024
Accepted: 12/08/2024
Published: 16/08/2024

ABSTRACT

Orthodontic treatment can lead to significant changes in the oral microbiome, potentially resulting in dysbiosis associated with increased risk of gingivitis and caries. The complex hard-soft tissue structures in the mouth harbour a diverse bacterial community, with around 700 different species identified. Orthodontic appliances, such as fixed braces, can alter the oral environment, leading to changes in the composition and abundance of oral microorganisms.

Studies have shown that fixed orthodontic appliances, including brackets and bands, can influence the colonization of periodontal pathogens like *Porphyromonas gingivalis*, *Prevotella intermedia*, and

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Cite as: *Maheshwari, Sandhya, Ameen Ashraf M P, Shariq Ahmad, and Saba Khan. 2024. "Orthodontic Appliances and Microbial Dysbiosis: Insights and Implications". South Asian Journal of Research in Microbiology 18 (8):41-51. https://doi.org/10.9734/sajrm/2024/v18i8381.*

Fusobacterium nucleatum, which are associated with periodontal diseases. These appliances can also increase the levels of cariogenic bacteria such as *Streptococcus mutans* and *Lactobacilli*, which are linked to dental caries.
On the other hand, clear aligners have been found to have a lesser impact on the oral microbiome compared to traditional braces, with some studies suggesting that they may even reduce the negative effects on periodontal health. However, clear aligners can still lead to changes in the oral microbiome, including increases in bacteria associated with gingivitis and periodontitis.
Overall, orthodontic appliances can significantly alter the oral microbiome, potentially leading to dysbiosis and increased risk of oral diseases. Proper oral hygiene instructions and monitoring are essential to minimize these risks during orthodontic treatment.

Keywords: Fixed orthodontic appliances; removable orthodontic appliances; cariogenic bacterias; periodontal pathogens.

1. INTRODUCTION

The mouth is home to around 700 distinct species of bacteria, making it one of the most diverse bacterial communities in the human body. The mouth has complex hard-soft tissue structures, such as teeth, the tongue, the gingiva, and the palate; depending on the different surface properties, distinct differences in the oral microbiota's structure can be seen [1].

Prokaryotes from about 700 different species have been found in the mouth cavity. Approximately 54% of these species have official names, 32% of the species are solely recognised as uncultivated phylotypes, while 14% are unnamed but farmed. These species belong to 12 phyla and 185 genera. There are 12 different phyla of bacteria: Firmicutes, Fusobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, Chlamydia, Chloroflexi, Spirochaetes, SR1 (Absconditabactria), Synergistetes, Saccharibacteria (TM7), and Gracilibacteria (GN02) [2].

2. FIXED ORTHODONTIC APPLIANCE

2.1 Periodontal Pathogens

Gingivitis (and periodontitis) development is influenced by a number of different factors. It has recently been demonstrated that bracket designs and material properties can affect the clinical parameters and bacterial profile [3,4]. The structure of orthodontic bands is complicated. They are made up of numerous stainless-steel parts that are joined together either by soldering or welding, and local irritation may be brought on by corrosion products and their effects on biofilms [5-7].

In one clinical research, individuals with chronic periodontitis receiving fixed orthodontic therapy experienced a significant decrease in the overall number of bacteria in the pocket; this could have been due to the materials employed, which altered the environment for biofilm growth. The placement of orthodontic appliances had an impact on clinical indicators and the colonisation of periodontal bacteria that are pathogenic, such as P. gingivalis, P. intermedia, P. nigrescens, and F. nucleatum [8-10].

In order to examine and contrast the oral microflora between orthodontic recipients and individuals in good health, Fubo Sun et al. used amplification of the 16S rRNA V3 region, analysis by PCR-DGGE, and quantifying of dominant species by real-time quantitative PCR [11]. The majority of the amplified bands that were chosen, eliminated, and sequenced for taxonomic identification belonged to Firmicutes and proteobacteria. Each group had Streptococcus and Neisserria species, but only those receiving orthodontic treatment had Pseudomonas, Veillonella, and Burkholderia species. This was consistent with another investigation that found opportunistic Pseudomonas species in orthodontic recipients and attributed this to Pseudomonas aeruginosa's superior ability to cling to dental surfaces over Streptococcus pneumonia [12]. According to a recent study, orthodontic patients had considerably higher levels of Pseudomonas species in addition to higher levels of coliforms such Enterobacter, Acinetobacter, and Yersini [13].

Additionally, Slots et al. identified Pseudomonas bacteria in the subgingival microflora of individuals with advanced adult periodontitis [14]. Patients with orthodontic issues have also had opportunistic infections such Veillonella, Neisserria, and others. According to Kim et al and Moon et al [15,16], Veillonella has also been discovered in a plaque that present subgingivally

of the of Korean patients who cigarette smoke and have severe periodontitis, while Burkholderia spp. have been discovered in samples of dentine caries [17].

Proteobacteria make up a large portion of the typical fora found in the gut and mouth cavity, whereas Actinobacteria play a significant role in the ambient microbiome. Actinobacteria significantly decreased with the use of fixed orthodontic appliances, according to earlier research[18]. According to certain research, saccharibacteria rise with ageing and have a part in the development of periodontitis [19,20].

The number of organisms from the genus Actinobacillus, Actinomyces, Corynebacterium, Kingella, and Neisseria as well as the species Haemophilus parainfluenzae, Lautropia mirabillis, and Rothia dentocariosa decreased noticeably in plaque samples [21]. According to several research, orthodontic treatment significantly increases the plaque level of Actinomyces.

After the appliances were taken out, Actinomyces naeslundii were more, according to Koopman et al.'s research on the genus Actinomyces. According to Tanner et al., Actinomyces is connected to gingivitis brought on by orthodontic therapy [22]. Neisseria has been associated with a group of orthodontic patients who have mild gingivitis, and it has been observed that its prevalence increases twelve weeks after removal and declines as removal approaches [18,22].

Three months after the initiation of orthodontic therapy, T. forsythia showed a sizable increase, according to the systematic analysis conducted by Gou et al., yet the information was mainly concerned with plaque that is subgingivally [23]. The results of Isamu Kado's thorough examination and inquiry strongly suggested that the usage of appliances for orthodontics increased the amount of the genus Tannerella in the oral cavity.

According to Zhao et al., despite employing the NGS (Next Generation Sequencing) approach, there was a significant decline in Prevotella abundance and no change in the composition of the overall microbial community in saliva. Patients with orthodontic treatment who had plaque at white-spot lesions showed a considerably higher detection rate for Granulicatella elegans [22], in contrast to research by Tanner et al, conducted by PCR, which stated the saliva notably included less of the genus Granulicatella.

These changes indicate that facultative anaerobic and aerobic microbes were replaced in the oral microbiota, namely in plaque, following the placement of fixed orthodontic devices, by obligate anaerobes and periodontopathogenic bacteria.

2.2 Cariogenic Bacteria

Since S. mutans and Lactobacillus are important pathogenic bacteria, O'Reilly reported that demineralization was seen a month after bonding in WSLs and dental caries. S. mutans and Lactobacillus, however, have been shown to significantly increase from month three to month six of orthodontic therapy, according to Topaloglu and Chang et al, [24,25]. However, Kupietzky et al. and Jurela A, et al. have shown that there was no discernible difference between the levels of S. mutans and other bacteria within the first three months [26,27]. However, the bulk of WSL or dental caries observations have been made after 2 years of orthodontic therapy, therefore bacterial modifications may have gone unnoticed in studies that lasted for a shorter period of time.

Over the course of the 18-month treatment, S. mutans prevalence increased considerably only in patients wearing conventional braces, while the proportion of S. mutans in the SLB group stayed at a significantly lower level with no change. Pellegrini et al. and Akin et al. both reported the same findings in their studies [28,29].

According to Jing et al.'s studies, lactobacillus levels somewhat increased, which is in line with Peros and Lara-Carrillo et al.'s findings. In a systematic review study, Lucchese et al. [30,31] noted that the use of orthodontic appliances had an impact on the increase in numbers of Lactobacillus and S. mutans [32].

A cross-sectional investigation, Klaus et al. found that poor oral hygiene was substantially associated with a greater incidence of Candida spp. in plaque and saliva. The two primary species discovered were Candida albicans and Candida dubliniensis. In 100% of salivary samples and 91% of plaque samples, respectively, S. mutans and Lactobacilli were both found to be bacteria.[33]. Topaloglu-Ak et al. carried out a comparable investigation on the use of fixed and detachable devices for the

cultured identification of salivary S. mutans, Lactobacillus spp., and C. albicans. Six months following the installation of fixed/removable appliances, they discovered a statistically significant rise in S. mutans and Lactobacilli, as well as a higher presence of C. albicans in the fixed appliance group than in the detachable appliance group [34].

Andrucioli et al. [35] examined bacterial contaminants following 30 days of premolar bands left in place following 16 months of fixed orthodontic therapy by utilising checkerboard DNA-DNA hybridization. S. mutans and Streptococcus sobrinus were more prevalent among the cariogenic species compared to Lactobacillus acidophilus and Lactobacillus casei.

According to Maria et al.'s study, there was no discernible change in the species of streptococcal bacteria between the orthodontic and healthy groups [36].

2.3 Self Ligating Bracket

According to research by Peter et al. [37], as compared to Elastomer appliances, Self-ligating appliances promote decreased oral microbial retention, including streptococci. The majority of patients' teeth with SL attachments displayed a reduced amount of ATP bioluminescence and fewer bacteria in plaque than teeth attached to brackets with elastomer, Jing et al. [6] found a substantial rise in S. mutans in individuals with traditional brackets.

When compared to conventional brackets ligated with stainless steel ligatures, Baka et al. and Uzener et al. did not find any statistically significant differences, but they did discover an increase in gram-negative and gram-positive bacteria (mainly Streptococci and Lactobacilli) [7.38].

2.4 Ceramic Bracket

The amounts of P. nigrescens, Actinomyces odontolyticus, T. forsythia, Actinomyces naeslundii, Capnocytophaga ochracea, Actinomyces israelii, and cariogenic bacteria like S. mutans and L. acidophilus were also very similar on metallic and ceramic brackets isolated from both front and back teeth, according to Anhoury et al.

Most significantly increasing species in ceramic ones were Selenomonas noxia, Capnocytophaga showae, and E. corrodens. Actinomyces gerencseriae, Streptococcus constellatus, and Streptococcus sanguis counts all considerably increased in anterior ceramic brackets

2.5 Ligature Wire vs Elastomeric Module

In comparison to incisors ligated with steel wires, archwires with elastomeric rings contained more microbes, according to research by Forsberg et al, [39] examined that how microbial plaque retention around fixed appliances were ligated, with steel ligatures and elastomeric ties affected the area.

After conducting a split-mouth investigation, Türkkahraman et al. [40] found that elastomeric rings caused greater bleeding at the teeth than steel ligatures did. T. forsythia and P. nigrescens were found in significantly larger concentrations at elastomeric ligatures, according to Alves de Souza et al. [41], but there were no appreciable differences between P. gingivalis, A. actinomycetemcomitans, and P. intermedia.

2.6 Molar Bands and Tubes

With the aid of 16S rDNA microarray and denaturing gradient gel electrophoresis (DGGE), Ireland et al. [42] discovered that T. denticola and P. nigrescens were on the rise while A. actinomycetemcomitans was on the decline. Plaque connected to both types of molar attachments had higher concentrations of P. gingivalis, T. forsythia, and E. nodatum, but interestingly only bonded molars were used to produce C. rectus, Parvimonas micra, A. odontolyticus, and V. parvula.

Mártha et al.'s DNA-strip method [43] was used to determine if subgingival plaque contained periodontopathogen bacteria. F. nucleatum was the most prevalent bacterial species across all groups and periods. Then, Capnocytophaga species (C. gingivalis, C. ochracea, and C. sputigena) and E. corrodens.

2.7 Labial vs Lingual

In their comparative investigation between the biofilm formation on the labial and lingual bracket surfaces, Yener et al. discovered that the biofilm accumulation on the lingual orthodontic therapy surface was more than that on the labial orthodontic therapy surface. For labial and lingual brackets, the locations with the largest biofilm buildup are the gingival, mesial, and distal

surfaces [44]. On the other hand, Sfondrin et al. did not discover any appreciable variations in clinical periodontal markers or microbiological results between buccal and lingual brackets [45].

Using the chequerboard DNA-DNA hybridisation approach, Gujar et al. assessed and compared the degree of appearance of orange and red microbial complexes in individuals receiving orthodontic treatment with aligners, traditional metallic fixed labial appliances, and lingual fixed appliances. They discovered that the lingual appliance had greater percentages of T denticola, Porphyromonas gingivalis, and Fusobacterium nucleatum. The study found that lingual fixed appliances exhibited greater microbiological contamination than labial fixed appliances, then with aligners . Fusobacterium periodontium and Prevotella intermedia were found in higher percentages in the labial fixed appliance [46]. According to Demling et al.'s investigation, the relative prevalence of Aa and Pg did not alter when fixed lingual appliances were inserted without supportive dental prophylaxis, although clinical characteristics specific to the lingual sites worsened [47].

3. REMOVABLE ORTHODONTIC APPLIANCE

Zharmagambetova et al. evaluated the effects of orthodontic treatment with ROA on 12-year-old individuals with dentoalveolar abnormalities in the oral microbiota. They discovered that C. albicans, S. aureus, and S. mutans frequency increased and the normal level of the microbiota reduced [48].

According to research by Arendorf et al, there is a clear connection between the usage of ROA and the occurrence of Candida [49].

After employing the ROA for 4 weeks, Marisela et al. revealed that the ROA and the supporting oral mucosa contained S. aureus, P. aeruginosa, and Candida spp. S. aureus was the most abundant bacterium in both the supporting oral mucosa and the ROA. This bacterium has a significant death rate and is connected to respiratory tract illnesses. P. aeruginosa is one of the most important lung infections and the leading cause of mortality as well as morbidity in cystic fibrosis patients. The results revealed that P. aeruginosa was the second-highest prevalent bacterium in the supporting oral mucosa and the ROA.

Additionally, Batoni et al. discovered that children receiving removable orthodontic appliances had a higher number of mutans streptococci [50]. According to Sumi et al., acrylic bases can increase the risk of pharyngeal colonisation and aspiration pneumonia by acting as a reservoir for respiratory infections [51].

A potential direct linear association between the existence of ROAs, C. albicans, and salivary PH levels was declared by prior investigations [52,53]. Although C. Albicans levels significantly increased as a result of ROAs, C.Dublininsis levels did not statistically significantly rise, according to Farrokh et al. [54]. This outcome is consistent with numerous studies that found C. albicans yeast to be highly and significantly prevalent in orthodontic patients.

Full-length 16S rRNA gene sequencing was employed in a study by Fernanda et al. to examine biofilm adhering to acrylic retainers on platforms created by ONT (Oxford Nanopore Technologies) for the V1-V9. In every sample they found, the phyla Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria were highly represented. Six phyla were identified in this study: the Firmicutes (Streptococcus, Gemella, Eubacterium, Selenomonas, Veillonella and related ones), the Actinobacteria (Actinomyces, Atopobium, Rothia, etc.), the Proteobacteria (Neisseria, Eikenella, Campylobacter and related ones), the Bacteroidetes (Capnocytophaga, Porphyromonas, Prevotella, etc.), the Fusobacteria (Leptotrichia and Fusobacterium) and the phylum of TM7. The most firmicute phyla are found on the plaque on the retainer. When there are a lot of Firmicutes in the mouth, polysaccharide hydrolysis has started. Furthermore, Firmicutes are also very important in the connection between gut bacteria and health. Firmicutes play a critical role in the capacity of the human body to take in fats and break down lipids, which is essential to staying healthy. The most common genus found clinging to acrylic retainers was likewise found to be Streptococcus. Actinomyces has been found to be the most prevalent bacterial species associated with dentures, contrary to earlier studies by Shi et al. [55]

In their report on pathogenic bacteria, they also mentioned Propionibacterium propionicum, Gordonia bronchialis, Campylobacter gracilis, Campylobacter nucleatum, Prevotella loescheii, Capnocytophaga granulosa, and other Porphylomonas species [56].

According to Alessandra et al.'s systematic review, Candida colonies grow during the first month of treatment, particularly those of the C. albicans species, and then they start to decline after a few months. The major rise in S. mutans occurs in the first 15 days of treatment and continues over the first few months. The microbiological count of Lactobacillus spp. rises over the first few months of treatment. During the first month of treatment, Moraxella catharralis and S. epidermidis levels considerably rise. During the first 6-7 months of treatment, Spirochaetes spp. considerably rises. Prevotella nigrescens, Aa, Pg, and Tf were not found following treatment [57].

3.1 Clear Aligners

Zhao et al. used 16S rRNA to examine the microecology of saliva and found that the use of clear aligners had no discernible effect on the microbial diversity of saliva [58].

In their work, Dong et al. discovered that over varying lengths of aligner usage, the diversity and constitution of the microbiome underwent significant changes at the phylum, order, genus, and species levels. There had been more Firmicutes than at the onset of the treatment at the phylum level. After 24 hours, Lactobacillales and Bacteroidales were abundant at the order level. Actinomycetales were first abundant in large numbers but then substantially declined. Streptococcus, Haemophilus, and Porphyromonas had higher genus-level abundances from T0 to T24 h, whereas Rothia, Lautropia, and Actinomyces had lower abundances. Streptococcus infantis' species-level abundance increased after 24 hours, but that of Streptococcus anginosus and Rothia dentocariosa more in comparison to initial stage [59].

However, as aligner usage length increased, Actinomyces abundance dropped. This may be because the inside surface of aligners did not provide favourable environment for colonisation or because the observation period used in this study was too short to allow for microbial growth [60].

Also In this study, Streptococcus and Lactobacillus, two key microbiome components associated with acid production and tooth caries, rose in abundance between 0 and 24 hours. The findings of this study reveal that as aligner usage length increases, the stability of the core microbiota declines, indicating the possibility of an unhealthy environment developing on the inner surface of aligners.

Despite the fact that patients receiving CAT had their teeth and gingiva covered for almost the entire day, Miethke et al. found that periodontal risk was much lower in those receiving the treatment [61]. The use of clear aligners may lessen the negative effects of orthodontic treatments on periodontal health, according to a study by Rossini et al. [62].

According to Kabilan et al. [63], the microbial community in saliva fluctuated between days 7 and 14, with the abundance of certain taxa altering considerably between days 7 and 14. The clear retainer held the most numbers of Streptococcus species from the Firmicutes phylum at both 7 and 14 days. This findings by Yan et al. are supported by data.[58] In the investigation by Kabilan et al., compared to saliva, Granulicatella increased by almost 2 log at day 7. However, in a different study by Tanner et al[22], Granulicatella elegans was found in plaque at a much higher level in a group of orthodontic patients with white-spot lesions.

The amount of parascardovia, which is generally isolated from dental caries, was significantly higher in the retainer as compared to saliva at 7 days. A gram-positive, functionally anaerobic cocci called Gemella has the potential to induce infective endocarditis; at all time intervals, bacteremia was also markedly elevated in the clear retention. Actinomyces decreased with rising retainer usage, mirroring the findings of Yan et al. This might be the case because Actinomyces would not thrive in this environment.

Additionally, the largest rates of Firmicutes, Bacteroidota, Proteobacteria, Actinobacteriota, and Fusobacteriota were found in the samples, which is similar with Kado et al.'s [21] findings. They also found that Actinobacteria levels on the retainer and in the saliva were consistent from 7 to 14 days, while the latter's levels were marginally higher than those on the clear retainer.

At 7 and 14 days, genus-level findings of gingivitis-related bacteria, including Solobacterium, Parvimonas, and Selenomonas, in the supragingival plaque, were also made in the retainer. At both 7 and 14 days, the retainer biofilm contained Tannerella and Fusobacterium,

which have the potential to induce periodontitis [64].

According to research by Qian et al. [65], the Invisalign group was shown to have a lower abundance of Firmicutes than the fixed appliance group while being similar to the control group. This finding suggested that the Invisalign group was more like a control group. According to the findings, Neisseria was more common at the genus level in the fixed appliance group than the Invisalign group. Neisseria colonises tooth surfaces quickly, and some studies have found a link between Neisseria and better dental health or less gingivitis [66].

3.2 Retainers

After orthodontic treatment with fixed appliances, Eroglu et al. compared and evaluated salivary microbial levels and periodontal status in patients using a fixed lingual retainer, a removable vacuum-formed retainer, or a Hawley retainer. They discovered that salivary S mutans and L casei levels and periodontal status do not differ between fixed and removable orthodontic retainers [67]. In addition, Bowen et al. compared three different types of retainers and discovered that, after six months of usage, the Hawley retainer outperformed the vacuum-formed retainer and the fixed lingual retainer in terms of Pg and Aa content and periodontal clinical characteristics [68].

Fixed retainers may increase cariogenic and periodontal infections and damage oral health, according to a study by Dhuha et al, A substantial rise in Streptococcus mutans, Lactobacillus acidophilus, Aggrigatibacter acti-nomycetemcomitans, Fusobacterium nucleatum, and Candida albicans was observed [68]. According to Kabilan et al's study, wearing a transparent retainer may cause alterations to the enamel or damage of periodontal tissue, particularly after 14 days of use [69].

3.3 Treatment Strategies and Clinical Implication

Using various orthodontic appliances can alter or enhance the oral microbiota, which increases the risk of developing periodontal diseases, caries, and white spot lesions.In the brief time after orthodontic appliance placement, it has been discovered that caries-causing bacteria such as Streptococcus and Lactobacillus are proliferating. Patients receiving orthodontic treatment are more likely to get halitosis if certain bacteria are growing. All of these raise the possibility that a patient may not be practicing proper dental hygiene.Treatment methods to avoid these include encouraging and educating patients about maintaining good hygiene, teaching them how to brush and floss properly, prescribing fluoride mouthwash for patients who are at risk of dental caries, using proper bonding and banding techniques and removing excess bonding material from the edges of braces and bands, and providing instructions on how to clean and take care of removable orthodontic appliances. Punnisa et al. found that combination therapy, which included brushing and Polident Pro Guard & Retainer®, was the most efficient way to remove retainer biofilms [70]. Furthermore, because Polident Pro Guard & Retainer® did not change the homeostatic balance of the bacterial populations attached to the acrylic retainers, it was safe to use.

4. CONCLUSION

Orthodontic treatment causes profound alterations in the oral bacterial environment that are linked to gingivitis and a higher risk of cariogenic responses. Giving patients oral hygiene instructions and monitoring their oral hygiene both before and during therapy are very important.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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