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Original Research

Prevalence of anaerobic microbiota in orthodontic patients – scoping review

Umarevathi Gopalakrishnan[1], Vidhya Selvaraj[2], Arvindh Kathir[3], Samala Abhinaya[4], Rajalakshmi Thakshinamoorthy[5]

[1]Professor, [2] Senior Lecturer, [3, 4, 5] Post graduate, Department of Orthodontics, Sri Venkateswara Dental College and Hospitals, Chennai

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Abstract

Introduction: Various appliances used in orthodontic treatment behave as plaque retentive sites which can harbor anaerobic microorganisms and this may be associated with a worsening of preexisting periodontal diseases or induce a variety of other conditions. There are contrary reports regarding the increased load of anaerobes during orthodontic treatment. This review aims to analyze the orthodontic literature regarding the prevalence of anaerobes before, during and after orthodontic treatment.

Objective: To analyze the literature on the prevalence of anaerobic microbiota and its relationship with orthodontics by using the keywords “anaerobes” OR “anaerobic microbiome” OR “red complex bacteria” AND “orthodontic” OR “fixed appliance”. The Pub med and Embase databases were searched till January 2022.

Results: Orthodontic treatment increases the prevalence of anaerobic microbiota especially the orange and red complex bacteria. The removal of orthodontic appliances has shown a significant reduction in plaque along with the corresponding anaerobic pathogens.

Conclusion: Proper maintenance of good oral hygiene during orthodontic treatment is essential to reduce the anaerobic microbial load, thus diminishing the risk of periodontal problems.

Keywords: Anaerobes; Red complex bacteria; Orthodontic appliance;

Address for Correspondence:
Umarevathi Gopalakrishnan, MDS
Professor, Department of Orthodontics, Sri Venkateswara Dental College and Hospitals
Chennai – 600103,
Email: gopkr_uma@yahoo.com

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INTRODUCTION

Oral micro biota has more than 700 microbial species consisting of eukaryotes, archaea, bacteria, fungi, and viruses living in specific ecological sites of the mouth namely buccal mucosa, keratinized gingiva, hard palate, tongue, tonsils, throat, saliva and sub and supra-gingival plaque. The environment present in the human mouth favors the growth of characteristic microorganisms. It provides a source of water, nutrients, moderate temperature, and anaerobic as well as aerobic environment [1]. They can be classified based on Gram staining as positive and negative, based on shapes as cocci and rods, based on oxygen requirements as obligate aerobes, micro aerophilic, facultative anaerobes and obligate anaerobes[2].Few anaerobic bacteria that are present in the oral cavity are *Bifidobacterium, Lactobacillus, Actinomyces, Propionibacterium, Treponema, Veillonella, Arachnia, Bacteroides, Eubacterium, Fusobacterium, Leptotrichia, Peptococcus, Peptostreptococcus, Selenomonas*species [3].

The malalignment of teeth tends to augment the plaque accumulation and hence the microbes as well. Orthodontic patients reported significant qualitative and quantitative differences in supra and subgingival plaque during the entire treatment period. Various appliances used in orthodontic treatment behave as plaque retentive sites which harbors periopathogenic or cariogenic bacteria. The virulence of bacteria depends on many factors, especially bacterial serotype and individual host susceptibility [4]. By increasing the plaque accumulation and deepening gingival sulcus, fixed orthodontic appliances can change the subgingival microbial environment [5]. Some studies have found that the content of periodontopathogens in the subgingival plaque of orthodontic patients was significantly altered [6]. Sub-gingival micro biota causing periodontitis is color-labeled in red, orange, yellow, green, and purple complexes. Dr. Sigmund Socransky developed the "complex theory" where periodontal pathogens are categorized based on their association with the severity of disease. In the complex theory, periodontal pathogens have been identified and classified by color to indicate which bacteria are associated with the onset and progression of periodontal disease. Early colonizers are Yellow, green and purple complexes, which are able to adhere with their fimbriae to the dental film, thus favoring the subsequent co-adhesion and co-aggregation of the bacteria of the orange complex. The orange bacteria are the "bridge species" that connect early colonizers and late colonizers like the red bacteria. They produce toxins and enzymes responsible for the progressive loss of attachment and increase in pocket depth, thus creating a hospitable environment in the gingival sulcus/pocket for living conditions and colonization by red-complex bacteria. The latter is the "late colonizers", lodged in the deepest pockets and strongly associated with bleeding in the advanced stages of periodontitis. Periodontal damage by red bacteria is the endpoint of a process during which different green/yellow and orange bacteria accumulate and co-aggregate, making the sub gingival niche a hospitable habitat for the red bacteria [7].

Anaerobic bacteria are not only responsible for periodontal issues; some of the bacteria are also capable of causing corrosion of metallic appliances[30, 31] in which case they become even more clinically significant since the usage of metal brackets is still prevalent in orthodontics.

There are several studies which tried to find the prevalence of these anaerobic bacteria in orthodontic patients. The aim of this review is to determine whether there is an increase in the prevalence of this yellow, orange and red complex anaerobic species in orthodontic patients compared with normal individuals.

MATERIALS AND METHODS

We used the search engines Pub med and Embase for the literature review in order to collect the articles that were published between Dec 1980 and January 2022. The key words were, "anaerobes and orthodontics",

"prevalence of anaerobes", "red complex bacteria", "orthodontic appliance" and "prospective studies". The selection was based on inclusion and exclusion criteria. We employed the PRISMA guidelines for this process.

Inclusion criteria:

Articles studying the prevalence of anaerobic organisms

Articles dealing with prevalence in orthodontic patients

Articles in English language

Articles between Dec 1980 and Jan 2022

Exclusion Criteria:

Articles in non-orthodontic patients

Articles on aerobic micro-organisms

Articles on non-human subjects

Data collection:

A customized data form was prepared which included the Author Name/Year of publication, samples and groups, sampling sites, the methodology used to assess bacterial prevalence, organism assessed and their inferences. To eliminate subjective bias, two independent observers were employed to study the articles and fill the forms. The final form was based on consensus opinion.

Data analysis:

Authors performed Qualitative analysis based on the information obtained from the customized data collection forms. The focus was on the organism assessed, methodology and percentage of prevalence.

Table I: Table representing the collected data

S.no	Study	Sample size	Groups	Sampling Sites	Method	Organism Assessed	Inference
1	Anhoury et al, 2002 (8)	28 orthodontic patients metallic brackets -32 ceramic brackets -24	At the day of debonding.	Two brackets from each patient	DNA probes	Td, Aa, Fn, ssvincentii, Sa, En, Ec, Cs and selenomonas noxia	Higher mean counts of Td, Aa, Fn, ssvincentii, Sa, and En.On metallic brackets while higher counts of Ec, Cs and selenomonas noxia on ceramic brackets.
2	Ristic et al 2008 (9)	32 orthodontic patients	Before bonding of fixed appliances (T0), 1 (T1), 3 (T2) and 6	Subgingival dental plaque samples	Culture	Pi, Aa and the group of other black-	Total number of microorganisms increased from T0 to

			(T3) months after the beginning of orthodontic therapy			pigmented anaerobes such as Pg and Fn	the maximum obtained in T2 recording time. Both clinical and microbiological values decreased 6 months after the beginning of orthodontic therapy
3	Thornberg et al 2009 (10)	190 orthodontic patients	At the beginning of orthodontic treatment (T1), at 6 months (T2), 12 months (T3), more than 12 months (T4) of treatment and 3 months after removal (T5).	Subgingival plaque	DNA probe analysis	Aa, Pg, Pi, Tf, Fn, Td, Ec and Cr	Pathogen counts increased significantly after 6 months of treatment The risk of having high counts of Pi, Tf, Fn, Td, Ec and Cr was significantly greater
4	Choi et al, 2009 (11)	30 Orthodontic patients and 30 control	2 weeks before appliance removal (T1) 3 months after appliance removal(T2)	Subgingival plaque 21,26,31,36	16 S rRNA-based PCR	The prevalence of Aa, Tf, Cr, Ec, Pi, Pg, Pn and Td	Tf at T1 is higher (26.7 %) than that of gingivally healthy control subjects (7.5%). The frequency of positive sites at T1 and T2 was 65% and 43.3% for Cr, and 53.3% and 30.8% for Ec, respectively.
5	Carrillo et al 2010 (12)	34 patients	Before starting orthodontic treatment and 1 month after.	Saliva and supragingival plaque	Culture	S.mutans, lactobacillus	A slightly increase of colony formation, after placement of appliances
6	Liu et al 2011 (13)	48 orthodontic patients Group A - 28 subjects at the beginning of orthodontic treatment Group B - 20 subjects at the end of orthodontic treatment.	before and after appliance placement in group A and before and after appliance removal in group B.	Subgingival plaque	Real-time qPCR	Pg	The level was high at the end of orthodontic treatment, and they decreased significantly after appliance removal
7	Topaloglu et al	69 patients who used removable and fixed	Baseline and at the 1, 3 and 6 month	Saliva samples	Culture	S.mutans, Lactobacillu	S mutans and Lactobacillus spp.,

	2011 (14)	orthodontic appliances	periodic controls			s spp., and C. albicans.	counts increased significantly 6 months after the insertion of appliance. C albicans presence was noted after 3 months
8	Kim et al 2012 (15)	30 orthodontic patients	Before placement of orthodontic appliances (T1), and 1 week (T2), 3 months (T3), and 6 months after placement of orthodontic appliances (T4).	Subgingival microbial samples 21,26,31,36	16s rRNA-based PCR	Aa, Tf, Cr, Ec, Pg, Pi,Pn and Td	Frequency of Tf, Cr, and Pn significantly increased after placement of orthodontic appliances. Cr and Pn appear to colonize immediately after the placement of orthodontic appliances, whereas Tf requires a longer time to colonize.
9	Yi Liu et al 2013 (16)	102 patients 57 cases of gingivitis patients with orthodontic appliances, 25 cases of periodontitis patients and 20 cases of periodontally healthy people			16S rRNA-based PCR and a multiplex PCR	Pg	Prevalence of Pg and rag locus genes in periodontitis group was the highest among three groups followed by orthodontic gingivitis group and healthy people
10	Ireland et al 2013 (17)	24 orthodontic patients	During treatment and up to 1 year after appliance removal	Plaque samples from the molars and upper lateral incisors	16S rRNA microarray		Pg, Tf, and En, while C.rectus, Parvimonasmicra and A. odontolyticus were also elevated with bonds.
11	ŽivkovićSandić M. Et al.2014 (4)	Group A : at the beginning Group B: at the end of orthodontic therapy.	Group A: before placement appliance (T1), after one month (T2), and after 3 months (T3). Group B: before appliance removal (T1), after one month (T2), and after 3	Subgingival plaque samples were collected from the right upper incisor (U1) and right upper first molar (U6).	PCR	Pg, Aa, Tf, Pi	No variation in frequencies for 3 anaerobes and the decreasing rate of Pg during 3 months from the beginning of orthodontic treatment

			months (T3).				
12	Ping liu et al 2014 (18)	169 patients 55 orthodontic patients with gingivitis, 49 gingivitis patients without orthodontic treatment 35 periodontitis patients and 30 periodontally healthy people		Subgingival biofilm samples	PCR	Fuscobacterium	The detection rate of Fn in periodontitis group and non-orthodontic gingivitis group was higher than the other two groups (p,0.01) while it was higher in orthodontic gingivitis group than in health people (p,0.05)
13	Vico et al 2015 (19)	122 patients 61 orthodontic and 61 normal individual	At baseline (orthodontic patients T1) and 10 days after bracket removal (T2).	Subgingival plaque samples	PCR	Aa, Tf, Td, Pi and Pg	The Aa and Pi organisms occurred in some subjects, irrespective of placement of bands. A decreased prevalence of Aa, Tf, Td, Pi 10 days after removal of appliance,
14	Klaus et al 2016 (20)	75 Orthodontic patients 25 patients each (good oral hygiene (GOH), poor oral hygiene (POH), and poor oral hygiene with white spot lesions (POH/WSL))		Saliva and plaque samples	Culture	Prevalence of Candida spp., Streptococcus mutans, and Lactobacilli	Candida prevalence in dental plaque of 60.9 % and in saliva of 73.4 % of the patients. High counts of S. Mutans and Lactobacilli in POH or POH/WSL patients
15	Martha et al 2016 (21)	25 orthodontic patients Group A: 15 patients who received orthodontic bands on first permanent molars Group B: 10 patients	Before bands and tubes application and 4–7 weeks after placement.	Subgingival sample	DNA-strip technique	Aa, Pg, Pi, Tf, Td, Pm, Fb, Cr, En, Ec, Cc	After one month of orthodontic attachment placement Ec, Pm, Td and Tf (Group A) and capnocytophaga spp.(Group B) showed greater prevalence

		with directly bonded tubes on the labial surface of the same teeth.					
16	Guo et al 2016 (22)	One hundred and eight malocclusion patients	Before and after treatment	Subgingival plaques	Quantitative real-time PCR	Pg, Fn, Pi and Tf	The detection rates of Pg, Fn, Pi and Tf increased from baseline to third month without significant difference, and then returned to pretreatment levels 12 month after applying fixed orthodontic appliances
17	Pan et al 2017 (23)	Group A: 61 orthodontic patients Group B: 56 periodontally healthy adolescents	After 1 month (T1), 2 months (T2), 3 months (T3), and 6 months (T4) in the case group and then compared with those of the controls	Subgingival plaque samples were obtained from the lower incisors.	16s rRNA-based PCR and fimagenotypes specific PCR	Pg	Maximum values were reached at 3 months after placement and the levels were decreased after 6 months
18	Sun et al 2018 (24)	30 orthodontic patients and 20 normal individuals		Saliva samples	PCR	Streptococcus and Pseudomonas species	Pseudomonas, veillonella and burkholderia species were present only in orthodontic patients, while streptococcus and neisserria species were present in both groups
19	Shirozaki et al 2020 (25)	28 orthodontic patients	T0: before orthodontic treatment; T1: at 6 months; and T2: 12 months post treatment.	GCF	Checkerboard DNA-DNA hybridization	Levels of 40 bacterial species, and of 3 cytokines (IL-1β, MMP-8, and TNF-α)	Red complex pathogens were in significantly greater proportions in T2 compared with T0
20	Kado et al 2020 (26)	71 orthodontic patients	Supragingival plaque samples: before placement (T0) and six months after placement (T1).	Supragingival plaque and saliva samples	16S rRNA meta-sequencing	Microbes	Capnocytophaga, Fusobacterium, and Leptotrichia spp., were more relatively abundant in supragingival

			Saliva samples at (T0), (T1) and then when appliance removal (T2).				plaque than in saliva. Conversely, Neisseria and Haemophilus spp. Were more abundant in saliva. Relative abundance of Prevotella, Porphyromonas, Capnocytophaga, Parvimonas and Selenomonas spp., were significantly higher in 6 months
21	Lemos et al 2020 (27)	17 orthodontic patients	At baseline and after 12 months of treatment	Subgingival biofilm samples	Checkerboard DNA-DNA hybridization Culture	40 bacterial species	Significant reduction in the mean proportions of the Actinomycesspps., and an increase in the orange complex species. The proportions of the red complex species remained unchanged. Level of Pi had increased in 12 months ($p>0.05$).

RESULTS

Search results

A total of 314 studies were obtained from PubMed, Embase and Google Scholar. After reviewing by 2 independent investigators, 86 articles were eliminated for duplicity. After screening of abstracts, 40 studies proved to be potentially eligible for full-text evaluation. After excluding the articles that are not relevant to our study, 24 Articles were included in the study. The flowchart of the literature search is presented in Fig. 1.

Description of studies:

Pertaining to the sample collection of the studies, 4 studies were conducted before and during orthodontic treatment (Ristic et al 2008), (Carrillo et al 2010), (Kim et al 2012), (Shirozaki et al.2020)[9,12,15,25], 7 studies during orthodontic treatment (liu et al 2011), (Topaloglu et al 2011), (Klaus et al 2016), (Martha et al 2016), (Guo et al 2016), (kado et al 2020), (Lemos et al 2020) [13,14,20,21,22,26,27], 3 studies during and after orthodontic treatment (choi et al, 2009), (ŽivkovićSandić M. et al.2014), (Vico et al 2015) [11,4,19] and one study (Thornberg et al 2009) [10] was conducted before, during and after orthodontic treatment.

Both orthodontic and non-orthodontic patients were included in 5 studies (Choi et al, 2009), (Yi Liu et al 2013), (Ping liu et al 2014), (Pan et al 2017), (Sun et al 2018) [11, 16,18,24]. Two studies (Anhoury et al, 2002), (Ireland et al 2013) [8, 17] compared different types of brackets used in the orthodontic treatment.

Sampling sites and methods

Samples were collected from different sites in different studies and the sites include supragingival plaque, sub gingival plaque, saliva, plaque from the brackets and gingival crevicular fluid (GCF).

Description of outcome

Prevalent periodontopathogens among the included studies

Early colonizers

Purple – *Veillonella parvula*[24], *Actinomyces odontolyticus*[17].

Green –*Capnocytophaga*[21,26], *Eikenellacorrodens*[8,10,11,15,21]

Blue – *Actinomyces spp*[4,8,9,10,11,15,19,21,27]

Middle or bridge colonizers

Orange complex bacteria –

Campylobacter rectus[15,21], *Eubacterium nodatum*[8,17,21], *Fuscobacterium nucleatum*[8,9,10,18,22], *Prevotella intermedia*[4,15,19,21,22,27], *Prevotella nigrescens*[11,15]

Late colonizers

Red complex bacteria - *Tannerella forsythia* [10,11,15,17,21,22], *Porphyromonas gingivalis*[9,10,11,13,19,21,22,23], *Treponema denticola*[8,10,19,21,18,24]

The microbial changes after orthodontic appliance placement

Short term (< 3 months) changes

In most of the included studies, total number of microorganisms like *Prevotella intermedia*, *Actinomyces spp* and the group of other black-pigmented anaerobes such as *Porphyromonas gingivalis* and *Fuscobacterium nucleatum* increased from the onset of treatment till 3 months after the treatment. In a study conducted by Topaloglu et al in 2011, revealed the increase in the count of *Candida albicans* after 3 months of placement [14]. *Campylobacter rectus* and *Prevotella nigrescens* appear to colonize immediately after the placement of orthodontic appliances, while *Tannerella forsythia* requires a longer time to colonize [15].

Long term (< 6 months) changes

Thornberg et al detected the microbial changes throughout the treatment term and found that the number of patients with high periodontopathogen counts increased 6 months after orthodontic appliance placement but then returned to the pretreatment level after 12 months [10]. In contrast to this, Kim et al., reported that the level of *Tannerella forsythia* remained at a high level over the first six months, without an obvious decrease. This might have resulted from short period of observation [15].

Changes after removal of orthodontic appliance

All the studies demonstrated that there was a decrease in the levels of the microbial load after the removal of orthodontic appliance. A study by Vico et al 2015, showed a decreased prevalence of *Actinomyces spp*, *Tannerella forsythia, Treponema denticola, Prevotella intermedia* even within 10 days after removal of appliance [19].

Difference between orthodontic and non-orthodontic population

Prevalence of *Porphyromonas gingivalis* and rag locus genes in periodontitis group was the highest, followed by the orthodontic gingivitis group and healthy people (Yi Liu et al 2013) [16].

Discussion

The results obtained from the included studies regarding the prevalence of anaerobic organisms and the changes in periodontopathogens during orthodontic treatment showed an overall increased tendency. After the placement of orthodontic appliances, all the studies reported an increasing tendency, except one study. But the microbial changes that occurred during orthodontic treatment were transient, as they tend to decline after several months of appliance placement or after the removal of the appliance. Polymerase chain reaction (PCR) was the most common method used among the included studies.

A study conducted by Anhoury et al, differentiated the predominant species between metallic and ceramic brackets. They found higher mean counts of *Treponemadenticola*, *Actinomycesspp, Fuscobacterium nucleatum, Actinomyces vincentii* on metallic brackets while higher counts of *Eikenella corrodens*, *Capnocytophaga* and S*elenomonas noxia* on ceramic brackets [8].

Subgingival plaque was the predominant site of collection followed by saliva, supra gingival plaque and GCF. A study conducted by kado et al., revealed a marked difference between the changes of microbial flora among plaque and saliva samples, collected from same individuals. *Capnocytophaga, Fusobacterium, and Leptotrichia spp*., were relatively more abundant in supragingival plaque than in saliva. Conversely, *Neisseria and Haemophilus spp.*, were more abundant in saliva [26].

Placement of attachments also imparts a difference in the periodontal pathogens. Martha et al., 2016 (15) study showed greater prevalence of *Eikenella corrodens, Prevotella nigrescens, Treponema denticola, Tannerella forsythia* in a group with band attachment and *capnocytophaga spp.*, in a group with tube attachment [15].

Fixed appliances promote plaque accumulation, which is the critical aetiological factor of periodontal disease. Moreover, sub gingival microbial composition is influenced by supragingival plaque accumulation [28]. Orthodontic tooth movement, including intrusion and tipping, can also move supragingival plaque into the sub gingival sulcus, and thus affect the sub gingival microorganisms. Apart from this plaque accumulation, metal corrosion, host immunity, hormonal levels and the microbial baseline of participants also affects the level and the content of microorganisms in sub gingival plaques during orthodontic treatment [29-32].

High counts of *Streptococcus mutans* and *Lactobacilli* were noted among the orthodontic patients with poor oral hygiene than the patients with good oral hygiene Klaus et al 2016 [17].

The clinical relevance of our review is that though the anaerobes increase during orthodontic treatment, the effect on gingival or periodontal status seems to be temporary since the levels of bacteria decreased after removal of appliances. The limitation of the current review is that, only 9 studies have a control group of healthy individuals to compare the level of microorganisms with that of orthodontic patients and sample size.

Conclusion

Our review concludes that the levels of anaerobic periodontopathogens temporarily increased after placement of an orthodontic appliance. After several months of application/removal of the appliance, the levels decreased or even returned to the pretreatment levels. This review emphasizes that orthodontic treatment might not permanently induce periodontal disease by affecting the level of sub gingival periodontal

pathogens. Regular periodontal examinations and good oral hygiene should be the top priorities for orthodontic patients, especially at the early stages of treatment. Further studies are required to assess the microbial changes throughout the orthodontic process.

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