ORIGINAL RESEARCH

"Red-complex Bacteria in Patients with Plaque-induced Gingival Enlargement" Undergoing Fixed Orthodontic Therapy: A Cross-sectional Study

Sajid T Hussain[1], Jaideep Mahendra[2], Janani Muralidharan[3], Pavithra H Dave[4], Little Mahendra[5], Vivek Sharma[6]

Abstract

Aim: The present study compares the quantitative analysis of the red-complex bacteria (RCB) in patients undergoing fixed orthodontic therapy with and without plaque-induced gingival enlargement.

Materials and methods: Forty subjects undergoing orthodontic therapy were categorized into two groups: control group: 18 periodontally healthy subjects without plaque-induced gingival enlargement undergoing orthodontic treatment and study group: 22 subjects with plaque-induced gingival enlargement undergoing orthodontic treatment. Plaque index (PI), bleeding on probing (BOP %), simplified oral hygiene index (OHI-S), and probing pocket depth (PPD) were recorded. The type and frequency of tooth brushing were also assessed. Plaque samples were collected from the periodontal pockets and analyzed using real-time polymerase chain reaction (RT-PCR) for the detection of *Treponema denticola (T.d)*, *Tannerella forsythia (T.f)*, and *Porphyromonas gingivalis (P.g),* and statistical analysis was performed.

Results: PPD, PI, OHI-S, BOP, type, and frequency of tooth brushing were higher in patients with plaque-induced gingival enlargement as compared to the controls. A significant increase in the levels of *T.d, T.f, and P.g* was found in both the groups, however it showed a statistical insignificance difference. Overall *T.d* was found to be more prevalent than *T.f* and *P.g* in both the groups. On correlating the RCB with the other variables. A positive correlation was seen with *T.d* and the oral hygiene index scores in the controls and the presence of *T.d*, *T.f,* and *P.g* with BOP and PPD in the study group.

Conclusion: The expressions of RCB levels in the dental plaque of patients undergoing orthodontic treatment with and without the inflammatory enlargement were more or less the same however, *T.d* was found at higher levels than the other bacteria.

Clinical significance: The study shows the association of the BOP and PPD and the presence of RCB in orthodontic patients with inflammatory enlargement putting these patients at a higher risk for periodontal attachment loss.

Keywords: Bacteria, Orthodontic treatment, Periodontitis, Plaque.

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Introduction

The oral cavity being the natural habitat of microbes promotes the formation of distinct microbial biofilm around the teeth. A delicate balance between the microbial colonies and the host response is usually maintained during the normal defense mechanism. According to the currently accepted hypothesis, periodontitis is a chronic infectious ailment of bacterial origin induced by periodontal bacteria which plays an important role in the origin and development of periodontal diseases.[1] Many endogenous and exogenous factors are responsible for the interaction between periodontal tissues and the primary infectious stimuli.[2] Diseases of periodontium often begin as gingivitis affecting the adolescent and adult population. It is common that during the same adolescent period, individuals may undergo orthodontic treatment with fixed appliances for the correction of malocclusion. These orthodontic appliances also create an additional retentive area and space that promote the adherence of microorganisms and growth of biofilm and this co-existence demands the higher requirements for oral hygiene maintenance in the oral cavity.[3,4] Moreover on comparison of various types of orthodontic brackets, literature reviews have stated that the use of stainless steel brackets resulted in a higher degree of inflammation in comparison with ceramic brackets due to irritation produced by materials used for orthodontic bonding. The mechanical irritation caused by these bands and wires may result in improper oral hygiene maintenance which may further result in plaque accumulation and food impaction thus contributing to the pathogenesis of plaque-induced gingival enlargement. It has been also stated that lingual orthodontic therapy has contributed

[1]Department of Periodontics and Implantology, Sree Balaji Dental College and Hospital, Chennai, Tamil Nadu, India

[2–4]Department of Periodontics, Meenakshi Ammal Dental College and Hospital, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India

[5]Department of Periodontics, Maktoum Bin Hamdan Dental University College, Dubai, United Arab Emirates

[6]Department of Periodontics, Desh Bhagat Dental College and Hospital, Gobindgarh, Punjab, India

Corresponding Author: Jaideep Mahendra, Department of Periodontics, Meenakshi Ammal Dental College and Hospital, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India, Phone: +91 9444963973, e-mails: drjaideep.perio@madch.edu.in; jaideep_m_23@yahoo.co.in

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to higher degrees of gingival inflammation in comparison with the conventional labial orthodontic therapy owing to the reason that wider lingual brackets cause a compromised inter bracket distance thereby making the routine oral hygiene procedures very difficult to perform thus leading to the risk of plaque accumulation and gingival inflammation.[4]

Due to poor oral hygiene maintenance, patients with fixed orthodontic appliances tend to exhibit signs of gingivitis and plaque-induced gingival enlargement with the formation of pseudo pockets.[5,6] These appliances further create favorable conditions for colonization of subgingival plaque containing bacterial species thereby further aggravating their invasion into the underlying tissues leading to gingivitis and plaque-induced gingival enlargement that itself manifests as an increased tissue volume which may bleed easily on probing. The gingival enlargement that occurs during the course of fixed orthodontic treatment is mainly due to the retentive areas by these appliances for the accumulation of plaque and food debris thereby resulting in the formation of pseudo pockets, wherein there is no evident clinical attachment loss present but the enlargement itself can give rise to an artificially deep pocket that resolves on the treatment of gingival hyperplasia. If left untreated, this condition may further attribute to the development of periodontal disease.[7,8]

In the current study, we aimed to assess the periodontal status, type, and frequency of brushing and the quantitative assessment of the expression of RCB *(T. denticola, T. forsythia, and P. gingivalis)* in the sites with plaque-induced gingival enlargement during orthodontic treatment thereby substantiating the role of orthodontic appliances as a reservoir for the bacterial colonization further contributing to the development of gingival and periodontal disease. The secondary objectives were to correlate periodontal parameters, type, and frequency of tooth brushing with the RCB levels expressed in the sites with plaque-induced gingival enlargement while undergoing orthodontic treatment.

Materials and Methods

Study Design

The current study was conducted between January 2021 and December 2021 in Chennai, Tamil Nadu, India. Fifty adolescent and adult populations belonging to the age group of 15–30 years, undergoing fixed orthodontic therapy in the department of orthodontia, SRM Dental College, Chennai, India were recruited for the study. Out of 50 study participants, five study participants were excluded as they were smokers. Two study participants underwent periodontal therapy and were under medication and hence were also excluded. Three study participants did not give their consent to participate in the study. Finally, 40 study participants were selected based on the inclusion and exclusion criteria. The inclusion criteria included both male and female study participants falling within the age range of 15–30 years, study participants with Angle's Class I malocclusion being treated with fixed orthodontic appliance undergoing orthodontic therapy, having plaque-induced gingival enlargement (study group), study participants with Angle's Class I malocclusion being treated with fixed orthodontic appliance undergoing orthodontic therapy without plaque-induced gingival enlargement (control group). Study participants who were smokers, alcoholics, drug abusers, patients who had periodontal therapy 6 months prior to the study, pregnant and lactating mothers, and study participants with systemic disorders and patients not willing to participate in the study were excluded. Finally, 40 patients were selected and grouped as control group consisting of 18 periodontally healthy study participants without plaque-induced gingival enlargement undergoing fixed orthodontic treatment and the study group consisting of 22 study participants with plaque-induced gingival enlargement undergoing fixed orthodontic treatment. The study protocol was explained to all the subjects in detail and written informed consent was obtained from them. All the study participants were undergoing fixed orthodontic therapy using conventional metallic regular ligating braces for a duration not less than 6 months in the first half at the time of the commencement of this study. The research protocol was carried out in accordance with the Helsinki Declaration of 1975 revised in 2013. Institutional Review Board, SRM dental college Chennai, India approved the study (protocol no: SRMU/M&HS/SRMDC/507).

Parameters Assessed

The periodontal examination was carried out with the patient sitting in an upright position with light facilities in the dental chair by a skilled, experienced, and calibrated investigator under standardized conditions. The periodontal parameters assessed included PI, bleeding on probing (BOP %), simplified oral hygiene index (OHI-S), and PPD.

The PI was examined according to Loe and Sillnes.[9] The BOP was evaluated as the percentage of teeth that bled on probing and calculated as bleeding sites per tooth. Oral hygiene status was assessed using an OHI-S proposed by Greene JC and Vermillion JR[10] The PPD was calculated as the distance from the gingival margin to the base of the pocket and further evaluated as the mean of all six sites examined for the entire dentition. The current study also recorded the history of the type and frequency of toothbrushes from the subjects as a part of routine clinical history examination.

Plaque Sample Collection

Subgingival plaque samples were collected using sterile paper points and preserved in 5% ethanol containing Eppendorf tubes and stored under -80°C for the further identification of RCB.

Genomic DNA Isolation

From the collected plaque samples, the isolation of genomic DNA was performed according to the protocol described by Brezezinska-Blaszezyk et al.[11] The paper points were thawed with lysozyme followed by vortexing and incubating for a period of 15 minutes for 37°C. The paper points were then removed and centrifuged at 10000 RPM for a period of 10 minutes. Pellets were then suspended in 500 µL of wash buffer and the microbial DNA was isolated using a genomic mini kit (PureLink Genomic DNA Mini Kit, catalog number: K182002 Thermo Fisher, Polana). The cells were lysed with proteinase K and the obtained DNA extract was stored in 50 µL of prewarmed elution buffer at a temperature of –20°C until further evaluation. The stored DNA samples were measured by spectrophotometer for further analysis.

Real-time Polymerase Chain Reaction

The bacterial primers for the identification of RCB were developed using 16S rRNA as a reference which was specifically designed to target the tested microorganisms. The microbial DNA was assessed using SYBR green dye.

DNA template was added to 20 µL PCR reaction mixture and the final product was suspended in 96 well solid PCR plate and amplified using a real-time thermo cyclometer. The PCR results were demonstrated using 2% gel electrophoresis observed under a UV transilluminator in the gel documentation system working at a wavelength of 260 nm. Lower CT values corresponded to the elevated levels of RCB in the subgingival plaque samples (Table 1).

Statistical Analysis

The statistical analysis of this study was performed using a statistical package for social science (SPSS) version –13.0.

One-way ANOVA analysis was performed to compare the mean and standard deviation of periodontal parameters, type, and frequency of tooth brushing and RCB, assessed among the groups. The Pearson's correlation between the periodontal parameters, type, and frequency of tooth brushing with the expression of RCB levels in both the groups was performed and the level of significance was calculated. A *p*-value of <0.05 was considered as a level of significance.

Results

On comparing the periodontal parameters between the groups, the mean value for PI was found to be 0.70 ± 0.58 and 1.36 ± 0.25 for control and study groups respectively (*p*-value = 0.013). The mean bleeding on probing percentage in the study group was found to be 65.6% which was found to be higher than the control (40.5%) with *p*-value = 0.0001. On comparison of oral hygiene parameters, the mean value of OHI-S was found to be 1.83 ± 0.62 and 2.79 ± 0.58 in control and study groups respectively which were again statistically significant (*p*-value = 0.015). The mean values for PPD in control and study groups were found to be 2.11 ± 0.32 and 4.50 ± 0.51 respectively which reached the level of statistical significance (*p*-value = 0.001). Of the 40 individuals examined, all 22 subjects in the study group, reported the usage of regular toothbrush while in the control group, 13 subjects reported the usage of orthodontic toothbrush for effective teeth cleaning, and the rest five used regular toothbrush. On comparison of mean values for the type of toothbrush used between the groups, the study group reported a statistically significant difference (*p*-value = 0.018). Similarly, the mean frequency of tooth brushing was highly significant between the groups. (*p*-value = 0.003) of the 40 subjects examined, 18 subjects who fell under the control group brushed their teeth twice daily whereas, in the study group, all the 22 subjects brushed only once.

On comparing the mean values of RCB among the groups, *T. denticola* was found to be more prevalent as compared to the other bacteria. We used a real-time PCR assay in our study. CT level, as a measure of outcome in RT-PCR analysis, is inversely proportional to the amount of target nucleic acid in the sample (i.e., the lower the CT level, the greater the amount of target nucleic acid of the microorganism). In the current study, the mean CT values for the expression of all three microorganisms ranged from 22.87–26.91 in both control and study groups, which indicated that there was a significant rise in the levels of all three micro-organisms. However, *T. denticola* was found to be more prevalent than *T. forsythia* and *P. gingivalis* in both the groups with the lowest CT-value. When these microorganisms were compared between the groups, *T. denticola, T. forsythia, and P. gingivalis* levels were found to be more or less similar with an insignificant difference (Table 2).

A significant positive correlation was found between oral hygiene index and the levels of *T. denticola* (*p*-value = 0.016) in the control group and BOP and PPD with all the three microorganisms in the study group. The other variables such as PI scores, type, and frequency of toothbrush in both the groups did not correlate with the RCB levels (Table 3).

Table 1: Red-complex bacterial primers

Bacteria	*Primer sequence*
P. gingivalis	TGC AAC TTG CCT TAC AGA GGG ACT CGT ATC GCC CGT TAT TC
T. forsythia	AGC GAT GGT AGC AAT ACC TGT C TTC GCC GGG TTA TCC CTC
T. denticola	TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA

Discussion

The dentofacial discordance which is commonly known as malocclusion is a subject of significant stress occurring in individuals belonging to any population as it draws the most attention from other people in interpersonal interactions and communications.[12] Across the globe, the prevalence of malocclusion is considerably high in the European population followed by the African population. The prevalence of malocclusion in India was reported with wide variation with a significant range from 19.6–96.5%. Awareness for the maintenance of the aesthetic and at the same time rectification of malocclusion has become a major domain in the adult and adolescent Indian population.[13,14] Most adolescents are opting for orthodontic management using fixed orthodontic appliances due to rising aesthetic concerns. The major drawback of these fixed appliances is the accumulation of plaque due to the placement of orthodontic bands and brackets irrespective of the successful outcome of the treatment.[15,16] This can also cause an increase in the count of anaerobic bacteria which may be a risk for future periodontal disease.[17] Proximal cleaning in these patients is again a difficult process that further eventually leads to inflammatory gingival enlargement and requires a periodontal intervention for the removal of plaque.[18] The various preventive and treatment strategies for orthodontic treatment-induced gingival enlargement in recent times are periodontal surgical treatment such as gingivectomy, maintenance of good oral hygiene practices, and nonsurgical periodontal therapy. One of the most common soft tissue complications associated with fixed orthodontic appliances is inflammatory gingival enlargement induced by the accumulation of plaque that might lead to compromised orthodontic tooth movement and cause aesthetic and psychological problems.[19] Thus the present study aimed to evaluate the periodontal status, type, and frequency of toothbrush, and the levels of RCB (*P. gingivalis, T. denticola, T. forsythia)* in patients undergoing orthodontic treatment.

The present study included 40 subjects within the age group of 15–30 years with malocclusion undergoing fixed orthodontic therapy. Periodontal parameters such as PI, BOP, OHI-S, and PPD were recorded and the information regarding type and frequency of tooth brushing was collected from all the subjects. Sub-gingival plaque samples were collected for quantitative assessment of RCB from all the subjects.

Table 2: Intergroup comparison of periodontal parameters, type, and frequency of tooth brushing and red-complex bacteria

Variables	*Groups*	*Mean ± SD*	*p-value*
Plaque index (PI)	Control group	0.70 ± 0.58	0.013*
	Study group	1.36 ± 0.25	
Bleeding on probing (%)	Control group	40.5 ± 21.8	0.0001*
	Study group	65.6 ± 22.3	
Simplified oral hygiene index (OHI-S)	Control group	1.83 ± 0.62	0.015*
	Study group	2.79 ± 0.67	
Probing pocket depth (PPD)	Control group	2.11 ± 0.32	0.001*
	Study group	4.50 ± 0.51	
Brushing type (conventional/orthodontic)	Control group	Regular toothbrush (*n* = 5) 2.25 ± 0.21	0.018*
		Orthodontic toothbrush (*n* = 13) 8.06 ± 5.66	
	Study group	Regular toothbrush (*n* = 22) 11.75 ± 7.77	
		Orthodontic toothbrush (*n* = 0)	
Brushing frequency	Control group	Brushing once daily 0	0.003*
		Brushing twice daily (*n* = 18) 2.03 ± 0.68	
	Study group	Brushing once daily (*n* = 12) 1.63 ± 0.65	
		Brushing twice daily (*n* = 0) 0	
Treponema denticola	Control group	22.98 ± 0.80	0.763NS
	Study group	22.91 ± 0.68	
Tannerella forsythia	Control group	25.02 ± 0.74	0.974NS
	Study group	25.01 ± 0.97	
Porphyromonas gingivalis	Control group	26.85 ± 0.87	0.905NS
	Study group	26.9 ± 01.58	

One-way ANOVA used for analysis; *p*-value <0.05 is considered statistically significant*; *T. denticola, T. forsythia, P. gingivalis* = expressed as mean ± SD; NS Nonsignificant

The present study revealed a statistically higher PI score seen in the orthodontic patients with a plaque-induced gingival enlargement (study group) as compared to the control group which was significant. These results were in concordance with Sukhia HR et al. and Huser et al. who observed a significant increase in PI scores when compared with the baseline in patients undergoing orthodontic treatment.[20,21] The higher scores of PI were also found to be similar to the other studies done by a few authors who reported a positive increase in the amount of plaque and degree of gingival inflammation during the orthodontic treatment (Alexander, Boyd and Baumrind, Skold-Larsson et al., Sallum et al.).[22–25] The authors explained that the increase in the plaque and gingival bleeding index could be attributed to the plaque retentive conditions occurring as a result of orthodontic appliances (Huser et al.).[21] It is stated that orthodontic appliances act as a plaque retentive area and this further leads to gingival inflammation owing to the host-bacterial interaction within the plaque biofilm. Orthodontic appliances result in compromised oral hygiene maintenance thus paving way for the growth of periodontopathogenic bacteria. It is imperative to note that various studies in the past were performed after the orthodontic appliance removal however our study was conducted during orthodontic therapy.

Bleeding on probing was evaluated among the groups. The results showed a higher percentage of BOP with significant differences between the groups. This was in accordance with the research work done by Nikita et al., and Sinclair PM et al.[26,27] According to them, mechanical irritation could be one of the factors for the increase in bleeding on probing which may be due to orthodontic bands in contact with the gingival margin. The BOP may also be due to gingival irritation due to various bonding agents and cement around the orthodontic bands. This may further aggravate plaque accumulation which in turn may lead to increased BOP. However, studies done by Zachrisson and Alnæs, Alstad and Zachrisson, Huser et al. did not evaluate the inflammatory condition.[28,29,21] It has been proven that the signs of gingival inflammation are associated mainly with gingival and periodontal diseases (Page et al.).[30] Our findings suggest that the presence of fixed appliances with banded molars does influence the inflammatory condition of the gingiva.

Table 3: Group-wise correlation of periodontal parameters, type, and frequency of tooth brushing with the levels of red-complex bacteria in the plaque sample

Parameters		*T. denticola*	*T. forsythia*	*P. gingivalis*
Plaque index	Control group	0.128	0.168	0.160
	Study group	0.116	0.317	0.3022
Bleeding on probing (BOP %)	Control group	0.345	0.321	0.124
	Study group	0.002*	0.0016*	0.003*
Oral hygiene index	Control group	0.016*	0.413	0.307
	Study group	0.838	0.227	0.743
Probing pocket depth	Control group	0.212	0.124	0.333
	Study group	0.021*	0.000*	0.003*
Brushing type	Control group	0.178	0.903	0.146
	Study group	0.643	0.532	0.234
Brushing frequency	Control group	0.869	0.905	0.755
	Study group	0.343	0.512	0.754

Pearson correlation used for analysis; *p*-value <0.05 is considered statistically significant*; [NS] Nonsignificant

The current study findings revealed a statistically significant difference of simplified oral hygiene index scores between the control and study group compared (*p*-value = 0.015). The control group subjects without any inflammatory gingival enlargement maintained better oral hygiene as compared to the study group. This finding was in correlation with Atassi and Awartani et al. who stated oral hygiene as unsatisfactory in orthodontic patients.[31] In contradiction, studies done by Ajayi O et al., and Onyeaso et al. mentioned well-maintained oral hygiene in patients undergoing orthodontic treatment.[32,33] In our study, the good oral hygiene status observed in the control group (subjects without inflammatory gingival enlargement) may be due to the capability of these patients to undergo proper oral hygiene maintenance around their fixed appliances. Our results also stated that most of the participants (control group) maintained oral hygiene by brushing twice daily with an orthodontic toothbrush. These subjects observed good oral hygiene which could be due to frequent brushing and proper home care measures taken in oral health maintenance. It is stated that adequate oral hygiene instructions and information on the home care maintenance of the orthodontic patients undergoing orthodontic therapy is required to maintain psychological well-being and to prevent the progression of periodontal disease.[34]

Our findings revealed that the mean PPD, was found to be higher in the study group as compared to the controls, and this was found to be statistically significant (*p*-value = 0.01). The pseudo pockets with no clinical attachment loss were found to be higher in the individuals with inflammatory gingival enlargement undergoing orthodontic therapy. This was at par with the studies done by Gomes et al., and Alexander et al. who also obtained a higher PPD level in orthodontic patients.[35,22] The increase in PPD could be attributed to the presence of inflammatory gingival enlargement which in turn is a reservoir for the higher plaque accumulation and improper oral hygiene maintenance found in the study group and vice versa. It can be speculated that patients wearing orthodontic appliances who have poorly maintained oral hygiene can lead to permanent alterations in the subgingival area thereby potentiating the development of inflammatory gingival enlargement thus causing pseudo pockets.[36]

Our findings revealed that the type of toothbrush used has an influence on the removal of plaque and oral hygiene status. On comparing the mean values of the type of toothbrush used between the control and study groups, the orthodontic toothbrush used in the control group was higher than the study group and this was found to be statistically significant (*p*-value = 0.018). These results were similar to the studies done by Williams et al. who showed a small but significant superiority of the orthodontic toothbrush in relation to the removal of plaque on the buccal surfaces, although this seemed to be confined to the anterior teeth.[37] Similarly, a study was done by Kilicoglu et al. also reported a statistically significant increase in the elimination of plaque in upper premolars and lower anterior teeth in the group with an orthodontic toothbrush.[38] Hence in the current study, the orthodontic toothbrush used in the control group had a positive influence in maintaining good oral hygiene with no inflammatory enlargement (Fig. 1).

The current study revealed a statistically significant difference in the mean values of frequency of tooth brushing among the control and study group. Since both, groups were having malocclusion and were undergoing orthodontic therapy and hence were subjected to brushing twice daily as a part of oral hygiene instructions. This was in concordance with a study done by Abdul Dari Memon et al. who reported the same frequency of brushing in subjects undergoing orthodontic treatment.[39] He stated that not only the frequency of tooth brushing alone but an adequate patient's awareness about the oral hygiene practices and persistent practice of personalized dental care are important factors in oral hygiene maintenance. Hobson and Clark also observed that the efficacy of good oral hygiene is majorly committed to the patient's motivation.[34] In the present study, the control group subjects brushed twice daily and maintained good oral hygiene in comparison with the study group subjects and hence showed a significant result with regards to frequency of brushing.

The mean values of RCB (*P. gingivalis, T. denticola, T. forsythia*) compared between the control and study group did not reach the level of statistical significance with a *p*-value = 0.763, 0.974, 0.905, respectively. The bacterial levels were more or less similar in both groups. Though the PI scores were found to be higher in the study group, the RCB levels were more or less the same. It is stated that the virulence of the microorganisms and quality of plaque is much more attributable to the progression of the disease rather than the mere presence of the plaque. This

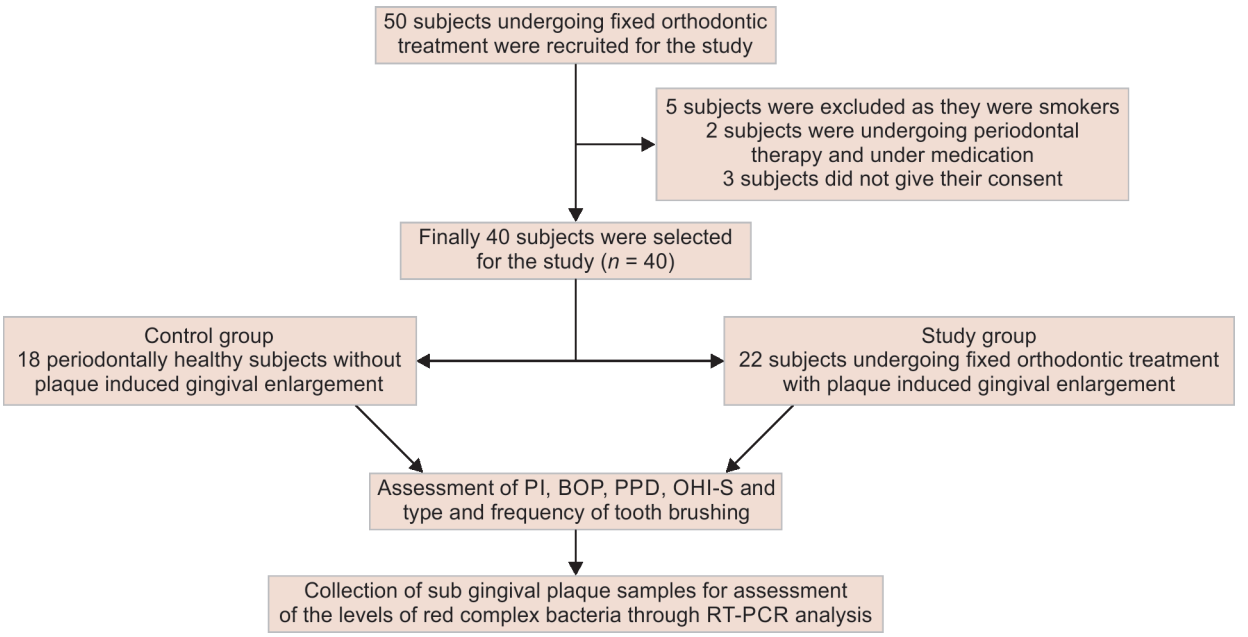

Fig. 1: Study flow chart

could be accredited to the presence of bonds and bands in the fixed orthodontic therapy which promotes plaque accumulation thus leading to colonization of RCB thereby marking a greater risk for gingival diseases (Table 2).

It has been stated that in patients with fixed orthodontic appliances, the preliminary changes in the microbial flora occur during the initial weeks of treatment.[16] It has been reported that the primary colonizers such as *Streptococci* and *Actynomicetes* species invade the tissues during the first month of treatment followed by orange complex species. After one month of orthodontic treatment, the levels of orange complex bacteria increase in the subgingival bacterial population thus accounting for 40% of the total bacteria counts. Finally, the RCB such as *T. denticola, P. gingivalis* and *T. forsythia*, significantly increase in the tissues during the first 3 months of orthodontic treatment resulting in subclinical gingival changes and gingival enlargement.[40] Hence it is critical to obtain the levels of RCB in patients undergoing orthodontic treatment so as to predict the gingival disease progression and onset of periodontal disease outcome. The microbiological results of the current study revealed an escalating increase in the levels of *T. denticola* as compared to *T. forsythia and P. gingivalis* in both the study and control groups. These results were in accordance with a study performed by Gong et al. were the author demonstrated a significant increase in the levels of the red complex species (*T. forsythia*, *P. gingivalis*, and *T. denticola*) in patients undergoing orthodontic treatment.[41] A.J Ireland et al. examined the microbiological changes in specific components of the plaque microbiota associated with orthodontic appliances and noted a significant increase in the counts of *T. denticola* initially during the first 3 months of orthodontic treatment.[42] Another study performed by Liu et al. reported an increase in the levels of *P. intermedia* and other orange complex species, while the proportions of the red complex species remained unchanged during the course of orthodontic treatment.[43] Thus, it can be stated that periodontal microorganisms play a vital role in the progression of the gingival and periodontal diseases, where orthodontic appliances are one of the major contributing factors acting as a plaque retentive area.

On correlating periodontal parameters, type, and frequency of tooth brushing with the levels of RCB in the plaque samples, the results of the current study revealed a highly significant correlation between oral hygiene index and the *T. denticola levels* in the control group thereby potentiating the role of this RCB in the development of gingival disease in patients undergoing orthodontic treatment irrespective of the presence of inflammatory enlargement. These study results were in accordance with Seung Mi Lee et al. who found a significant increase in the population of RCB including *T. denticola* in subgingival plaque of the patients undergoing orthodontic treatment (Table 3).[44] Hence it can be stated that a higher prevalence of *T. denticola* species could be a potentiating factor for the gingival inflammation occurring in orthodontic patients which could be attributed to the motility nature of the spirochete (Table 3). On correlating the periodontal parameters such as PPD and bleeding on probing with the levels of RCB, there was a positive correlation found in the study group with a statistically significant *p*-value (Table 3). These results significantly correlated with a study done by Eckley et al. who also found a significant positive correlation between an increase in PPD and the RCBl levels in the patients undergoing orthodontic treatment.[45] It is stated that the common clinical sequela of orthodontic therapy is gingival hyperplasia. This enlargement occurs as a result of excessive plaque accumulation thereby leading to an increase in the number of anaerobic RCB thus triggering an inflammatory process in the gingiva thus attributing to increased PPD. Moreover, the inflammation of gingiva results in ulceration of junctional epithelium thus contributing to increased probe penetration.

Overall, the current study gives a telescopic view on the prevalence of RCB in patients undergoing orthodontic treatment associated with inflammatory enlargement. Orthodontic treatment is one of the most important local modifiers of plaque formation as the placement of orthodontic brackets greatly alters the ecological

balance of the microbial community thereby potentiating the risk for the development of gingival and periodontal disease. The findings of the current study revealed the specific characteristic of the periodontopathogens present in the periodontal pockets of patients undergoing orthodontic therapy. Further evaluation of these microorganisms with regard to their interaction with the host environment in orthodontic patients should be performed in the future.

The current study also substantiates the fact that the patients undergoing orthodontic treatment require a rigorous plaque control regime to prevent gingival inflammation thereby maintaining optimal periodontal health. The various oral hygiene practices should be performed on daily basis by these patients in order to make sure that they maintain good oral hygiene as they are more vulnerable to gingival diseases. For reducing the further risk for gingival inflammation and pseudo pockets at the time of fixed orthodontic treatment, more awareness should be given to the type and characteristic of the orthodontic appliances in order to make them self-cleansing area even with normal tooth brushing. Care should be taken that these appliances should not pose a risk for plaque retention and at the same time, the frequent periodontal intervention for maintaining oral hygiene during the retentive period also becomes mandatory. These patients should be given proper oral hygiene instructions such as brushing twice daily and can receive some supplementary oral hygiene aids such as varnishes, gels, mouthwashes, or dentifrices. Care should also be taken for these to reduce the risk for oral ulcers.

The limitations of the current study included the lack of periodontal intervention in these subjects undergoing orthodontic treatment, its cross-sectional nature, and its small sample size. This study forms a base for further exploring the wide range of periodontal microorganisms in association with RCB as the ultimate bacterial load contributing to the gingival and periodontal diseases in patients undergoing fixed orthodontic treatment thereby planning a proper periodontal intervention and prevention in these patients.

Conclusion

This study revealed a unique fact that the expression of RCB in the dental plaque of patients undergoing orthodontic treatment was higher irrespective of the presence or absence of gingival enlargement. Hence, periodic nonsurgical intervention and thorough oral hygiene maintenance are mandatory in patients undergoing orthodontic treatment to prevent the risk of periodontal disease onset.

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