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Evaluation of the periodontal parameters and red complex microorganisms in Patients undergoing orthodontic treatment: A microbiological study

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ABSTRACT

Aim: The aim of this study was to evaluate the changes in the periodontal parametersand red complex microorganisms of the patients scheduled for fixed orthodontic treatment.

Materials and methods: Twenty-four subjects with malocclusion who were scheduled to undergo fixed orthodontic treatment were selected for the study based on the inclusion and exclusion criteria. Periodontal Parameters such as plaque index (PI),gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL) were assessed at each time point:at baseline that is before fixed orthodontic treatment, third month and sixth month during the course of fixed orthodontic treatment. Following the assessment of periodontal parameters, the subgingival plaque samples from (RAMFJORD TEETH) were obtained and collected after appropriate tooth isolation using a sterile Gracey curette with one single vertical stroke. The samples were then transferred into Eppendorf tubes containing 1ml phosphate buffer solution and stored in freezer at -200 C maximum temperature.After measurement of clinical parameters and sample collection, all the subjects underwent scaling and root planing. Oral hygiene instructions were given. They were then referred back to Department of Orthodontics and dentofacial orthopaedics for fixed orthodontic therapy. All subjects were recalled to the department of periodontology for assessment of clinical parameters and sample collection at each time point (3rd month and 6th month) while undergoing fixed orthodontic therapy.Scaling and root planing was done thereafter. Oral hygiene instructions were again reinforced.

Results: There was a gradual increase in the Plaque Index, Gingival Index, Bleeding on Probing, Probing Pocket Depth, Clinical Attachment Level and Red Complex Microorganisms, *Porphyromonasgingivalis, Tannerella forsythia* and *Treponemadenticola* from baseline to 3rd and 6th month of fixed orthodontic treatment.

Conclusion: The results of this study shows that orthodontic appliance favours Plaque accumulation, resulting in more inflammation and bleeding.Proper oral hygiene and a healthy periodontium should be maintained throughout the orthodontic treatment for a successful outcome .

Keywords: Fixed orthodontic treatment, Red complex microorganisms, Oral hygiene maintenance

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I. INTRODUCTION

Multidisciplinary approach is often necessary to treat complex dental problems and there cannot be a better example than ortho-perio interaction. Orthodontic treatment is based on the principle that if prolonged pressure is applied to a tooth, tooth movement will occur as the bone around the tooth remodels. This response of the bone is mediated by the periodontal ligament.1

A well-accepted rationale for orthodontic treatment is to prolong the life of the individual's dentition. Periodontics and Orthodontics are interlinked in many ways. A healthy periodontium will definitely lead to the success of any orthodontic treatment. Diseased periodontium with the periodontal pockets, bleeding and attachment loss can weaken the overall supporting structure of the tooth thereby leading to alveolar bone resorption and tooth loss.2Therefore it become mandatory for an orthodontist to check the periodontal condition before initiating the orthodontic treatment.In the recent times it has been seen that most of the orthodontic treatment failures are due to poor periodontal maintenance.3 Very limited studies in the past have evaluated the periodontium, both clinical and microbiological during the orthodontic therapy. Hence we aimed to evaluate the changes in periodontal parameters and red complex microorganisms during the course of orthodontic treatment.

II. MATERIALS AND METHODS

Twenty-fourpatients with malocclusion who were scheduled to undergo fixed orthodontic treatment and had visited the Out-patient Department of periodontics, Meenakshi Ammal Dental College and Hospital, Chennai for the periodontal evaluation and oral prophylaxis were selected for the study based on the inclusion and exclusion criteria. The study was approved by the "Institutional Review Board" MADC/IRB-IX/2016/154 MAHER -Deemed to be University, Chennai. The patients were explained about the study and written informed consent was obtained from those who agreed to voluntarily participate in this study. The inclusion criteria includedi)Patientswithin the age group 16 to 25 years ii)Scheduled to undergo fixed orthodontic treatment. History of any systemic diseases, use of antibiotics in the past three months, presence of periodontal disease, pregnancy,any periodontal procedure in past six monthsand smokers were excluded from the present study protocol.

III. CLINICAL EVALUATION OF SUBJECTS

Subjects underwent a brief case history recording which included patient's chief complaint, medical and dental history andintraoral examination.Periodontal parameters such as Plaque Index (PI), Gingival Index (GI), Bleeding on Probing (BOP), Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) were recorded at baseline, 3rd month and 6th month during fixed orthodontic treatment. Williams periodontal

probe was used for clinical examination and parameters were recorded to the nearest millimetre.

After the periodontal assessment, the samples for the identification of red complex microorganisms were obtained from subgingival areas of maxillary right molar, maxillary left central incisor, maxillary left premolar, mandibular left molar, mandibular right central incisor and mandibular right premolar (RAMFJORD TEETH) after appropriate tooth isolation from saliva. Subgingival plaque was removed using a sterile Gracey curette with one single vertical stroke. If any of the represented vital tooth were missing, the adjacent tooth was used instead. The samples were transferred into Eppendorf tubes containing 1ml phosphate buffer solution and stored in freezer at -200 C maximum temperature further analysis.

After the collection of subgingival plaque samples, all the subjects underwent scaling and root planing. Oral hygiene instructions were given and the patients were motivated to maintain good plaque control. They were then referred back to Department of Orthodontics and dentofacial orthopaedics for fixed orthodontic therapy. All subjects were recalled at 3rd month and 6th month during fixed orthodontic therapy. The periodontal parameters were again recorded and subgingival plaque samples were collected followed by scaling and root planing. Oral hygiene instructions were reinforced.

IV. STATISTICAL ANALYSIS

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 20.1 (IBM Corporation, Chicago, USA). Mean and standard deviation of all the parameters was estimated at different time points. Repeated measures ANOVA test was used to investigate either change in mean scores over various time points and level of significance was evaluated with $p \leq 0.05$.

V. RESULT

The mean plaque index on 0 day was 1.14±0.16 which increased to 1.53±0.30 in 3rd month and further increased to 1.81±0.39 in 6th month. The mean gingival index on 0 day was 0.91±0.27 which increased to 1.32±0.15 on 3rd month and 1.57±0.33 on 6th month. The mean bleeding on probing on 0 day was 40.5±21.7% which increased to 65.5±19.4% in 3rd month and further increased to 79.7±23.1% in 6th month. The mean probing pocket depth on 0 day was 2.21±0.60 mm which increased to 2.51±0.60 mm on 3rd month and 2.91±0.70 mm on 6th month. The mean clinical attachment level on 0 day was 2.21±0.60 mm which increased to 2.51±0.60 mm on 3rd month and 2.91±0.70 mm on 6th month. (Table 1)

On comparing the mean plaque index from 0 day to 3rd month, there was a change of -0.40±0.67 which was statistically significant (p=0.000). The mean plaque index from 0

day to 6th month was also found to be significant with a change of -0.67±0.08 (p = 0.000).Similarly, the mean plaque index from 3rd month to 6th month was statistically significant with a change of -0.28±0.04 (p = 0.000). The mean gingival index, from 0 day to 3rd month, also showed a change of -0.42±0.66 which was statistically significant (p = 0.000). A change of -0.66±0.10 and -0.25±0.05 was found in the mean gingival index from 0 day to 6th month and 3rd month to 6th month respectively which was statistically significant at p = 0.000.

On comparing the mean bleeding on probingbetween the varies time periods, a change of -25±3.8%, -39.1±4.9% and -14.1±2.3% was noted from 0 day to 3rdmonth, 0 day to 6th month, 3rdto 6th month respectively which was found to be statistically significant (p=0.000).On comparing the mean probing pocket depth between the varies time periods, there was a change of -0.31±0.64 mm, -0.70±0.08 mm, -0.40±0.72 mm from 0 day to 3rd , 0 day to 6th and 3rd to 6th month respectively which was statistically significant at p=0.000. On comparing the mean clinical attachment level between the varies time periods, there was a change of-0.31±0.64 mm, -0.70±0.08 mm, -0.40±0.72 mm from 0 day to 3rd month, 0 day to 6th month and 3rd to 6th month respectively which was statistically significant at p=0.000.(Table 2).

The gene expression levels of *Porphyromonasgingivalis* was expressed by its CT-values. The CT-value is inversely proportional to the level of gene expression. The decrease in CT-value indicated increase in the levels of *Porphyromonasgingivalis* in the plaque samples. On comparing the mean CT value of Pg from 0 day to 3rd month, 0 day to 6th month, 3rdto 6th month there was a change of 4.64±0.48, 8.07±4.11 and 3.43±0.44 respectively which was statistically significant (p = 0.000). Similarly, on comparing the mean CT value of Tf from 0 day to 3rd month, 0 day to 6th month, 3rdto 6th month there was a change of 4.065±0.36,7.38±0.51 and 3.31±0.47 noted which was statistically significant (p = 0.000). On comparing the mean CT value of Td from 0 day to 3rd month,0 day to 6th month and 3rd to 6th month, a change of 4.50±0.32, 7.09±0.31and 2.60±0.25 was also found to be present with astatistically significant difference from 0 day to 6th month and 3rd to 6th month at p = 0.000. However the change was found to be non -significant from 0 to 3rd month .(Table 3).

VI. DISCUSSION

Sometimes the orthodontic treatment may result in deterioration of periodontal health due to the accumulation of plaque in the retentive areas of the orthodontic brackets leading to various types of periodontal complications, such as gingival recessions, bone dehiscence, gingival invaginations and/or the formation of pockets.4Oral hygiene is greatly complicated following the placement of fixed orthodontic appliances. The greater plaque-retentive nature of orthodontic appliances causes plaque accumulation at the gingival margin and may contribute to increased incidence and severity of gingival inflammation.5 Monitoring gingival and periodontal health throughout the orthodontic

treatment and repeated reinforcement of acceptable oral hygiene routines have become an integral part of modern orthodontic practice.6

In the present study we aimed to evaluate the changes in periodontal parameters and red complex microorganisms during various phases of orthodontic treatment.A total number of 24 patients with malocclusion who were scheduled to undergo fixed orthodontics were selected for the study. The periodontal parameters were recorded and plaque samples were collected from the gingival sulcus for the estimation of red complex bacteria.

On comparing the periodontal parameters, the mean plaque score increased, inspite of scaling and root planing. (Table 1) This was in accordance with the studies done by Ristic M et al and Lo BA et al.7Maintaining good oral hygiene is a challenging task during fixed orthodontic treatment. Orthodontic bands, arch wires and brackets can act as plaque retentive factors.8In our study,inspite of good scaling and root planing the periodontal parameters were high and the presence of bacteria still prevailed due to the presence of orthodontic bands and brackets.The gingival index score was increased during the 3rd and 6th monthinspite of oral hygiene reinforcement. (-0.25±0.05). The mean change in gingival index at different time period was found to be higher. This was in accordance with the studies done byRistic M et al.9 This author stated that increased inflammatory response occurs due to increased plaque accumulation which in turn leads to increased gingival index score.The mean bleeding on probing at different time period was increased and found to be significant.This was in accordance with the studies done by Sinclair PM et al .10According to him, the increase in bleeding on probing was most likely caused by mechanical irritation due to bands, which are likely to be in contact with the gingival margin. Excess cement and composite during bonding and banding, may also lead to gingival irritation and increased plaque accumulation which in turn will cause increased bleeding on probing.10The mean probing pocket depth at different time period was found to be higher over a period at different time intervals. This was in accordance with the studies done by Naranjo AA et al.11The author stated thatthe mean increase in probing pocket depth might be due to pseudo-pocket formation or by deeper penetration of the probe into the weakened connective tissue. Pseudo-pockets can occur within 1-2 months of appliance placement and this occurs most commonly in the interproximal and posterior sites.This could be due to increased food impaction, poor oral hygiene and mechanical or chemical irritation.The mean clinical attachment level at different time period was also high. This was in accordance with the studies done byKarkhanechi M.12The author stated that during light orthodontic force movement,increased bone turnover around teeth occurs and PDL undergoes ischemia, which may increase the potential for a negative effect on the supporting bone and soft tissues.Theoretically, this may increase susceptibility to periodontal breakdown. Most studies evaluating the effect of tooth movement have shown that clinical attachment

loss will only occur in the presence of active inflammation due to periodontitis.12 (Table 2)

Subgingival plaque samples were collected at different time periods using curettes, scalers, paper points, broaches within cannulae and irrigation of periodontal pockets.13In our study, curettes were used to collect subgingival plaque sample. Curette sampling is an efficient technique to obtain plaque both in terms of quality and quantity. Curette can obtain sample from the entire pocket.

The subgingival plaque samples were analyzed for the presence of red complex organisms using PCR technique. PCR was chosen as a method for detecting the pathogenic microorganisms.14Some of putative pathogens such as *Porphyromonasgingivalis*, *Treponema* species are fastidious and difficult to culture. It has been shown that PCR is much more sensitive than bacterial culture and affords a better detection of microorganisms. Therefore PCR is a suitable and a very promising method for bacterial diagnosis.15
The gene expression levels of the three microorganisms were expressed by its CT-values. The CT-value is inversely proportional to the level of gene expression.16The decrease in CT-value indicates increase in the levels of microorganisms.

Microbial changes usually occurs within a short period of time after orthodontic therapy.17 Several authors have established the timeline for these microbial changes and found that within 12 days after orthodontic therapy is initiated, changes in bacterial composition begin to occur.18 At this time, a greater number of cocci and motile rods can be found in orthodontic patients compared to those without appliance. By 6 weeks, the amount of cocci decreases, whereas an increase in spirochetes and motile rods is noted.19 By 3 months, bacteria associated with the red and orange complex are established. In our study, there was an increase in red complex organisms over a time period. Shifts in the microbial composition due to orthodontic bands may be the result of decreased plaque control and moreover, the appliances themselves may also alter the microbial composition.20 (Table 3)
The present study demonstrated the clinical effect of orthodontic treatment on periodontal tissue. Periodontal condition in patients undergoing orthodontic treatment should be monitored carefully. The other treatment options like subgingival irrigation with chlorhexidine,electronic tooth brush and powered floss should also be considered for maintaining the healthy periodontium. Also, prior to the orthodontic treatment, an initial diagnosis and referral to control active periodontal disease should also be taken into consideration. Once the orthodontic appliances are placed, the patients need to be instructed about oral hygiene measures as every orthodontic intervention has a periodontal dimension. The interdisciplinary co-operation with clinical excellency in both the disciplines may transform patients with attractive esthetic dentition.

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VII. CONCLUSION

This study evaluated the periodontal parameters and red complex microorganisms during orthodontic treatment. There was a statistically significant increase in periodontal parameters like plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment level. The levels of*Porphyromonasgingivalis,Tannerella forsythia* and *Treponemadenticola* also increased statistically. The mean changes in the periodontal parameters and the levels of red complex bacteria at different time periods was also statistically significant.

Variables	0 day	3rd Month	6th Month
Plaque index	1.14±0.16	1.53±0.30	1.81±0.39
Gingival index	0.91±0.27	1.32±0.15	1.57±0.33
Bleeding on probing (%)	40.5±21.7	65.5±19.4	79.7±23.1
Probing pocket depth (mm)	2.21±0.60	2.51±0.60	2.91±0.70
Clinical attachment level (mm)	2.21±0.60	2.51±0.60	2.91±0.70

INDEX	Time period	Mean change	P-value

Plaque accumulation is favoured by the physical constitution of different parts of fixed orthodontic appliance.They also greatly reduce the efficacy of mechanical biofilm removal by tooth brushing and other mechanical aids. Hence it is necessary to provide periodic oral prophylaxis with oral hygiene instructions to the patients undergoing fixed orthodontic treatment.

TABLE 1: Mean and standard deviation of clinical parameters at 0 day, 3rd month and 6th month.

Table 2: Comparison of mean, standard deviation and Level of significance for clinical parameters at different time periods:

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		± SD	
plaque index	0 day-3rd month	-0.40±0.67	0.000(S)
	0 day- 6th month	-0.67±0.08	0.000 (S)
	3rd month-6thmonth	-0.28±0.04	0.000(S)
gingival index	0 day-3rd month	-0.42±0.66	0.000(S)
	0 day- 6th month	-0.66±0.10	0.000 (S)
	3rd month-6thmonth	-0.25±0.05	0.000 (S)
bleeding on probing	0 day-3rd month	-25±3.8	0.000(S)
	0 day- 6th month	-39.1±4.9	0.000(S)
	3rd month-6th month	-14.1±2.3	0.000(S)
probing pocket depth	0 day-3rd month	-0.31±0.64	0.000(S)
	0 day- 6th month	-0.70±0.08	0.000(S)
	3rd month-6th month	-0.40±0.72	0.000(S)
clinical attachment level	0 day-3rd month	-0.31±0.64	0.000(S)
	0 day- 6th month	-0.70±0.08	0.000(S)
	3rd month-6th month	-0.40±0.72	0.000(S)

S – S S-
S - Statistically significant | NS - Statistically not significant
Level of significance $p \leq 0.05$

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TABLE 3: Comparison of mean, standard deviation, mean change and level of significance for red complex microorganism at different time period:

Red Complex Microorganism	Time period	Mean change ± SD	P-value
Porphyromonasgingivalis	0 day-3rdmonth	4.64±0.48	0.000 (S)
	0 day- 6th month	8.07±4.11	0.000 (S)
	3rd month-6th month	3.43±0.44	0.000(S)
Tannerella forsythia	0 day-3rdmonth	4.065±0.36	0.008 (S)
	0 day- 6th month	7.38±0.51	0.000 (S)
	3rd month-6th month	3.31±0.47	0.000 (S)
Treponemadenticola	0 day-3rdmonth	4.50±0.32	0.244 (NS)
	0 day- 6th month	7.09±0.31	0.000 (S)
	3rd month-6th month	2.60±0.25	0.000 (S)

S - Statistically significant | NS - Statistically not significant
Level of significance $p \leq 0.05$

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