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ORIGINAL RESEARCH

ASSESSMENT OF PLAQUE RETENTION ON DIFFERENT ORTHODONTIC BRACKETS USING REAL TIME POLYMERASE CHAIN REACTION- A COMPARITIVE MICROBIOLOGICAL STUDY

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ABSTRACT:

Background: The aim of the study is to compare the effect of various bracket ligation systems and ceramic brackets with metal brackets on periodontal status in the patients undergoing orthodontic treatment that will be quantified in terms of plaque index ,gingival index, probing pocket depth at different time intervals of treatment along with a quantitative analysis of various microorganisms implicated to be most commonly present in dental plaque in patients undergoing orthodontic therapy using real time PCR. **Materials & methods:** Twenty patients seeking orthodontic treatment visiting the Department of Orthodontics were included in this study. All the subjects were informed about the procedures to be performed and signed informed consent was obtained from the parent/guardian. Patients were instructed to maintain their normal dietary habits and report back to the department after 3 weeks. The patients were randomly divided into two study groups. Each group consisting of ten patients. In Group I, self-ligating metallic brackets were compared with conventional metallic brackets. In Group II, self-ligating ceramic brackets were compared with conventional ceramic brackets. This comparison is carried out using split mouth study in which one type of bracket is used in maxillary right and mandibular left quadrant and the other type of brackets on maxillary left and mandibular right quadrant. Patients are recalled after 3 weeks and a clinical periodontal evaluation of patients is done in terms of plaque index, probing pocket depth and bleeding on probing before the bonding procedure was performed. The values were measured for all the teeth that are bonded except for the molars. All the teeth were evaluated at four sites per tooth (distofacial ,facial, mesiofacial and lingual). The clinical evaluation is followed by bonding using standardized isolation taking care that there should be no bonding agent left over anywhere around the bracket base. **Results:** Comparison of four types of brackets for S. mutans using PCR revealing that the increase in S mutans count among different bracket systems as statistically not significant (p value =0.5091). Comparison of four types of brackets for S. sobrinus using PCR revealing that the increase in S.sobrinus count among different bracket systems as statistically not significant (p value =0.5795). Comparison of four types of brackets for L.acidophillus using PCR revealing that the increase in L. acidophillus count among different bracket systems as statistically not significant (p value =0.2023). Comparison of four types of brackets for L.casei using PCR revealing that the increase in L. casei count among different bracket systems as statistically not significant (p value =0.7922). **Conclusion:** The microbiological counts for self ligating ceramic brackets was almost the same as conventional ceramic brackets Intra bracket comparison at different time intervals revealed a significant increase in bacterial count as well as the periodontal index for all the brackets.

Key words: Brackets, Orthodontic, Plaque

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INTRODUCTION

Orthodontic treatment these days involves a combination of bracket systems that provide the orthodontist with optimal technical performance as well as esthetic acceptability for the patient. To fulfill the esthetic

considerations, ceramic brackets were introduced in 1986 to substitute metal brackets ,which not only were esthetically pleasing but also helped the orthodontist to execute the desired treatment outcome with a standardized bracket system.[1]

Metallic brackets had certain limiting factors like the force decays and inadequate control over tooth movement when elastomerics are used for ligation, poor control over oral hygiene maintenance, increased chair side time for wire ligation, increased frictional values and failure to provide and maintain full archwire engagement.[2]

To surpass these, self-ligating brackets were introduced in 1990s. Self-ligating brackets had better full arch wire engagement, reduced friction between the bracket and the arch wire, faster arch wire removal and ligation and thus less chair side.[3]

However, orthodontic treatment is always associated with certain amount of risk especially when the treatment principles regarding the maintained of good oral hygiene are neglected that may result in questionable treatment outcomes after commencement of treatment.[3] The most common iatrogenic risk is enamel decalcification characterized by loss of inorganic tooth substance .This takes place when the pH of oral environment around the teeth falls below a critical value which will result in diffusion of calcium and phosphates out of the enamel. This early loss of enamel structure can be seen as opaque white spots.[4] Various orthodontic brackets along with archwires, elastics, bands and other attachments create a new retentive area that makes cleaning and access to plaque retaining areas difficult.[4, 5]

In addition, metallic brackets have the ability to induce certain changes in oral environment like acidic pH, increased plaque accumulation and further more decalcification. This is because of the hydrophobic, electrostatic and certain other interactions between metallic surface and the bacterias.[6]

The method of ligation used to attach arch wire to the tooth is an additional factor that can play an important part in development of dental plaque. It has been seen that teeth ligated with an elastomeric ring accumulates more plaque and thus more microorganisms than teeth ligated with steel ligature. This may thus predispose to development of dental caries and gingivitis in patients with unsatisfactory oral hygiene. However it has been observed that the bacterial adhesion is more with bonding adhesives than the bracket materials.[7- 10]

Other than enamel demineralization, associated with orthodontic therapy is the gingival inflammation and periodontal break down. Most of the patients undergoing therapy develop moderate generalized gingivitis that is usually evident after one to two months after placement of appliance. The increased pocket depth is the result of edematous gingival rather than any apical movement of gingival pockets.[11]

The plaque formation can be influenced by various factors like dietary composition, fluoride exposure, oral hygiene of patient and immune factor, it can also be influenced by the type of ligation used to attach the arch wire to the tooth .It was thus seen that teeth ligated with elastomeric rings have more chances of gingival bleeding then those ligated with steel ligature wire.[12- 14]

It has also been seen that self ligating brackets accumulate less plaque than elastomeric ligatures but comparatively more plaque than stainless steel ligature which is attributed to the clips and other retentive areas in the self ligating brackets.[15]

Thus far, so many studies have been done to evaluate the inter relation of enamel decalcification and methods of ligation. However, only a few studies have been done to find out the effect of various types of bracket ligation systems on periodontal status of the individuals undergoing orthodontic treatment.

The aim of the study is to compare the effect of various bracket ligation systems and ceramic brackets with metal brackets on periodontal status in the patients undergoing orthodontic treatment that will be quantified in terms of plaque index ,gingival index , probing pocket depth at different time intervals of treatment along with a quantitative analysis of various micro organisms implicated to be most commonly present in dental plaque in patients undergoing orthodontic therapy using real time PCR.

MATERIALS & METHODS

The clinical study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, Vokkaligara Sangha Dental College and Hospital (VSDCH), Bengaluru. Twenty patients seeking orthodontic treatment visiting the Department of Orthodontics were included in this study. All the subjects were informed about the procedures to be performed and signed informed consent was obtained from the parent/guardian. The study was approved by the institutional ethical committee and review board, Vokkaligara Sangha Dental College and Hospital, Bengaluru.

METHOD OF COLLECTION OF DATA

A preliminary examination of the patients who fulfilled the selection criteria was carried out followed by thorough supragingival scaling and polishing. After thorough oral prophylaxis, patients were given standardized tooth brush and tooth paste and were instructed to brush three times a day without using any other oral hygiene measure.

Patients were instructed to maintain their normal dietary habits and report back to the department after 3 weeks. The patients were randomly divided into two study groups. Each group consisting of ten patients. In Group I, self-ligating metallic brackets were compared with conventional metallic brackets. In Group II, self-ligating ceramic brackets were compared with conventional ceramic brackets. This comparison is carried out using split mouth study in which one type of bracket is used in maxillary right and mandibular left quadrant and the other type of brackets on maxillary left and mandibular right quadrant. Patients are recalled after 3 weeks and a clinical periodontal evaluation of patients is done in terms of plaque index, probing pocket depth and bleeding on probing before the bonding procedure was performed.

The values were measured for all the teeth that are bonded except for the molars. All the teeth were evaluated at four sites per tooth (distofacial ,facial, mesiofacial and lingual). The clinical evaluation is followed by bonding using standardized isolation taking care that there should be no bonding agent left over anywhere around the bracket base.

Brackets from same manufacturer were procured to prevent any bias caused by variation in the bracket structure and size. The conventional brackets were ligated with 0.010'' stainless steel ligature wire with 0.014'' NiTi wire used for initial alignment.

GROUP I

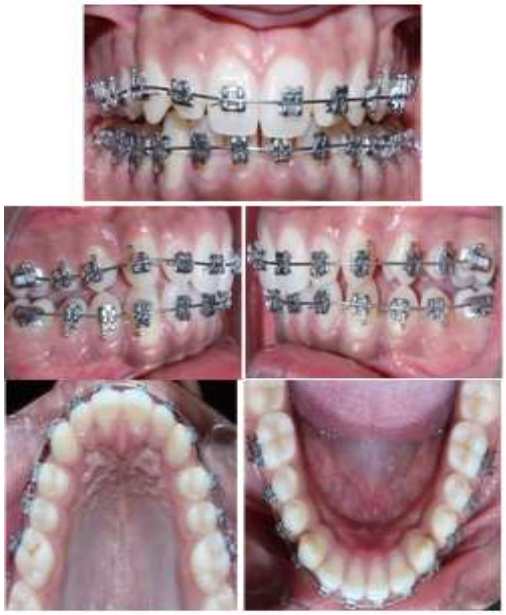

Figure 1: Bonding Done With 0.022'' Slot Mbt Metallic Brackets In Maxillary Right And Mandibular Laft Quadrant And Self Ligating Metallic Brackets In Maxillary Left And Mandibular Right Quadrant

GROUP II

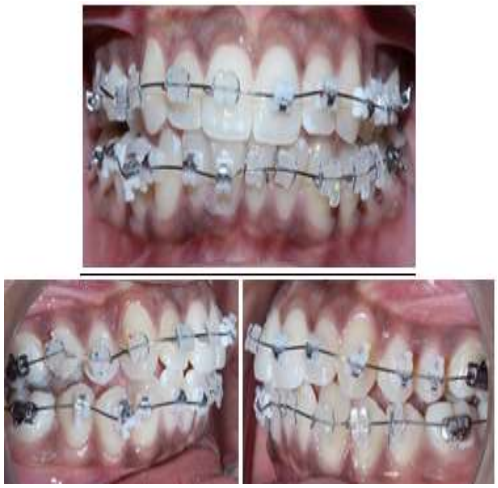

Figure 2: Bonding Done With 0.022'' Slot Mbt Metallic Brackets in Maxillary Right and Mandibular Left Quadrant and Self Ligating Metallic Brackets in Maxillary Left And Mandibular Right Quadrant

After bonding, patients are instructed to continue with the standardized oral hygiene procedures and to report the department back after 1 week. After one week of bonding, patients were again evaluated for the periodontal status including the same measurement index. It was made compulsory that the same clinician should check the periodontal status of all the patients to eliminate observer's bias. Patient is recalled after 3 months of bonding for next periodontal evaluation using same standardization performed before by the same observer. Before the clinical evaluation, supragingival plaque samples are collected from the entire isolated labial surface of lateral incisors using sterile curette and pooled in the eppendorf tube. Similarly supragingival plaque samples from labial surface of maxillary left lateral incisor were pooled in different eppendrof tube.

Clinical parameters that have recorded at baseline and 1 week, 3 months are:

1. Plaque Index (Silness and Loe, 1964)
2. Bleeding on probing(Ainamo and Bay)
3. Probing Pocket Depth (PPD)

The values were recorded for all bonded teeth, except for the molars, at 3sites per tooth. The periodontal evaluation was carried out by the same trained clinician using a periodontal probe. Real Time PCR allows the accumulation of amplified products to be detected and measured as the reaction progresses.

Chemistry:

1. DNA binding Dyes (SYBR Green I)
2. Fluorescently labelled sequence specific primers or probes(Taq Man).

Real Time PCR assay with Taq Man system is based on 5'-3' exonuclease activity of Taq Polymerase has been developed for quantification of DNA copy number. Oligonucleotide probe with a reported dye at 5' end and a quencher dye at 3' end is desiged to hybridize the target gene.Quencher dye is cleaved by 5'nuclease activity of Taq Man polymerase resulting in accumulation of reporter fluorescence. This release allows rapid detection and quantification of DNA. Specialised thermal cyclers equipped with fluorescence detection modules are used to monitor the fluoresvcence as the amplification takes place.This measured fluorescence reflects the amount of amplified product in each cycle. DNA extraction from the plaque samples was done using highly purified Invitrogen DNA isolation kit (Purelink™ DNA extraction kit). 1 mg of wet weight of paque is washed with phosphate buffered saline twice and the precipitate is suspended in 100µl of cell lysis solution and incubated with 20U of mutanolysin per millilitre and 0.2 mg of lysozyme per millilitreat 37ºC for 2 hours. The lysate is boiled at 100ºC for 10 minutes and chromosomal DNA is extracted. Oligonucleotide primers and probes designed from specific gene for S. mutans, S .sobrinus, L.acidoplilus, L.casei were used .

For each Real Time PCR 20µL of mixture containing 1µL of lysed cells ,1 Taq Man universal PCR master mix ,200 nM sense and antisense primer and 250 nM of Taq Man probe was placed in each wall of 96 well plates.

Amplification and detection is performed using QIAGEN with the following cycles:

- 50ºC for 2 minutes,
- 95ºC for 10 minutes,
- 60 cycles of 95ºC for 15 seconds
- 58ºC for 1 minute

RESULTS

This is an in-vivo study to compare the plaque retention on different orthodontic brackets. The aim is to compare the effect of different types of bracket ligature systems on plaque retention and quantitative analysis of Streptococcus mutans , Streptococcus sobrinus , Lactobacillus casei and Lactobacillus acidoplillus using REAL TIME POLYMERASE CHAIN REACTION. The objectives are to assess the amount of plaque retention using different types of brackets

- before bonding
- one week after bonding ,
- three months after bonding

Each parameter is given the corresponding statistical hypotheses, results and conclusions.

Table 1: Comparison 0f Four Types Of Brackets For S. Mutans Using PCR

Groups	Count	Sum	Average	Variance	Standard Deviation
SELF LIGATING METAL	10	32136.4	3213.64	31051686	5572.40
CONVENTIONAL METAL	10	-23916.3	-2391.63	2E+08	14133.37
SELF LIGATING CERAMIC	10	-32470.8	-3247.08	1.93E+08	13891.14
CONVENTIONAL CERAMIC	10	54	5.4	1946526	1395.18

Table 1 shows the comparison of four types of brackets for S. mutans using PCR revealing that the increase in S mutans count among different bracket systems as statistically not significant (p value =0.5091).

Table 2: Summary of Comparison for S. Mutans

ANOVA							
Source of Variation	SS	df	MS	F	P-value	F crit	**Not Significant**
Between Groups	2.51E+08	3	83757461	0.787	0.5091	2.866	
Within Groups	3.83E+09	36	1.06E+08				
Total	4.08E+09	39					

Table 2 shows the comparison of four types of brackets for S. sobrinus using PCR revealing that the increase in S.sobrinus count among different bracket systems as statistically not significant (p value =0.5795). Comparison of four types of brackets for L.acidophillus using PCR revealing that the increase in L. acidophillus count among different bracket systems as statistically not significant (p value =0.2023). Comparison of four types of brackets for L.casei using PCR revealing that the increase in L. casei count among different bracket systems as statistically not significant (p value =0.7922).

Table 3: Comparison Of Four Types Of Brackets For S. Sobrinus Using PCR

Groups	Count	Sum	Average	Variance	Standard Deviation
SELF LIGATING METAL	10	-39750.5	-3975.05	2.12E+08	14561.32
CONVENTIONAL METAL	10	6969.3	696.93	2212931	1487.59
SELF LIGATING CERAMIC	10	-34109.7	-3410.97	1.3E+08	11409.63
CONVENTIONAL CERAMIC	10	1244.5	124.45	116251.4	340.96

DISCUSSION

Oral cavity is an ecosystem rich in bacterial flora. Plaque is considered as an important factor in onset and progress of periodontal disease and caries. The development of dental plaque is dependent on several factors like patients diet, the composition of oral flora, maintenance of oral hygiene, nature of saliva. In orthodontic patients the malposition of teeth and the presence of orthodontic attachments and archwires create an ecological stress situation which alter the microbiological balance conducive for plaque accumulation.[13] Orthodontic appliances influence the nature of the dental plaque i.e. physical, chemical and biologic charecteristics33. Orthodontic fixed mechanotherapy bring about a drop in pH, increased carbohydrates, streptococci and lactobacilli.4 Studies show that orthodontic patients have increased plaque accumulation, in addition each mg of plaque contain greater concentration of bacteria and carbohydrate.[4] This plaque is highly cariogenic as the acid-producing bacteria is increased 4 and may result in transient (gingivitis) as well as permanent (white spot lesions) damage to the dentition.[16]

Studies have shown that the appliance type and design hinder effective cleaning of the surface of the enamel uncovered by the brackets.5 Among the orthodontic appliances, brackets are more prone to plaque accumulation because of their complex design. One of the factors being ligation method also contributes to this.10Concerning the method of ligation, a study done by Hakan Turkkahraman et al on archwire ligation techniques, microbial colonization and periodontal status in orthodontically treated patients concluded that teeth ligated with elastomeric rings had greater number of bacteria especially Streptococcus mutans and lactobacilli and bleeding on probing compared to teeth ligated with steel ligatures.[17-20]

Self-ligating brackets became popular in late 1990s that have hinged covers and facilitate elimination of elastomeric or stainless steel ligatures. This feature had the advantage of eradication of the cross-contamination, which may accidentally take place in the process of ligature handling and frequent change, and the claimed improvement in the oral hygiene of patients which is attributed to the fact that the patient can clean the surfaces because of reduced complexity and with less retentive sites for microbial colonization.[21] These are available as passive and active self-ligating brackets with claims of reduced friction, light forces, efficient sliding mechanics, and easy clinical application. These bracket systems differ with respect to clip properties, wire types, and sequences.[22- 25] Additionally, Pellegrini et al showed in an in-vivo study that teeth bonded with self-ligating attachments had fewer bacteria in plaque than did teeth bonded with elastomeric brackets.[26- 28] On the other hand, N Pandis and Zeliha Muge Baka in their studies with respect to periodontal status in self-ligating brackets concluded that self-ligating brackets are no better than conventional brackets.[29- 33]

Metallic brackets are known to have the highest critical surface tension and have increased risk for enamel demineralization seen as white spot lesions.[30] These white spot lesions significantly involve the labial surface of maxillary lateral incisor due to access to salivary flow and the distance between bracket and free gingival margin.[5, 6]

Because of the increasing awareness for more esthetic treatment options in young adults, especially women, the usage of tooth colored brackets made of ceramics is increasing. Bracket material is also considered as one of the main factors in plaque retention with studies showing ceramic brackets adhering less biofilm than the metal brackets in long-term thus appearing to exhibit advantageous material properties.[30] Various methods have been tried to quantify the bacterial count as accurately as possible including dark field microscopy[13], bacterial culture using selective media followed by stereomicroscopy or scanning electron microscopy.[4,8,10,14,19,22,24,27] Rapid adenosine triphosphate (ATP)-driven bioluminescence assays had also been used as a quantitative measure of microbial numbers in dental plaque. Bioluminescence assays measuring energy metabolites, including ATP, had shown to have high correlations with plaque mass obtained from both human subjects.[26]

The isolation and identification of the micro organisms in the above studies has been based on the colonial morphology grown on mitis-salivarius-bacitracin agar and the isolated colonies were then identified by biochemical, immunologic, and genetic tests. These laboratory procedures can be inaccurate, time-consuming, and laborious. Polymerase chain reaction (PCR) had been used to overcome these limitations as it is a simple, rapid and highly specific method using specific DNA fractions for the detection and identification of microorganisms.[23,25] Traditional method use Agarose gels for detection of PCR amplification at the final phase or end-point of the PCR reaction. However, Agarose gel results are obtained from the end point of the reaction thus is very time consuming and results may not be obtained for days. Thus ,it is difficult to quantify accurately the number of bacteria using conventional PCR because the reactions are evaluated after gene amplification is completed. In addition, the quantification of PCR products can be affected by contamination, interfering substances and unequal amounts of collected samples.[34- 38]

To overcome all these shortcomings and to quantify more accurately, Real time PCR had been introduced as it detects the accumulation of amplicon during the reaction. A real-time PCR assay with the TaqMan system based on the 5'-3' exonuclease activity of Taq polymerase had been developed for the quantitative detection of DNA copy number. An oligonucleotide probe with a reporter fluorescent dye attached to its 5'end and a quencher dye attached to its 3'end is designed to hybridize to the target gene. During PCR amplification, the quencher dye of the probe is cleaved by the 5' nuclease activity of Taq polymerase, resulting in the accumulation of reporter fluorescence. Rapid detection and quantification of DNA is accomplished by release of the fluorescent dye during amplification. The data is thus measured at the exponential phase of the PCR reaction while as traditional PCR methods use Agarose gels or other post PCR detection methods, which were not as precise. Thus, Real-Time PCR makes quantification of DNA and RNA easier and more precise than past methods.[37]

S mutans because of it is an acidogenic and aciduric behaviour is considered to be the primary organism responsible for enamel caries. This along with acid producing Lactobacillus species is commonly quantified for microbiological examination.[33, 38- 40]

The results of the study show that there was no statistically significant difference between conventional and self ligating brackets of either metal or ceramic type. The periodontal index and microbiological counts of ceramic brackets was lower than metallic brackets ,but was statistically insignificant. However, there was a significant increase in bacterial count as well as the periodontal index between the baseline and three months after bonding within the same bracket type.

CONCLUSION

The periodontal index for self ligating metal brackets was almost the same as conventional metal brackets. The microbiological counts for self-ligating metal brackets were almost the same as conventional metal brackets. The periodontal index for self ligating ceramic brackets was almost the same as conventional ceramic brackets but lesser (statistically insignificant) than metallic brackets of either type. The microbiological counts for self ligating ceramic brackets was almost the same as conventional ceramic brackets Intra bracket comparison at different time intervals revealed a significant increase in bacterial count as well as the periodontal index for all the brackets .

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