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EFFECT OF MODIFIED BASS BRUSHING TECHNIQUE AND HABITUAL BRUSHING ON THE CARRIAGE OF ORAL MICROBES IN PATIENTS WITH FIXED ORTHODONTIC APPLIANCES – A COMPARATIVE STUDY

Navneet Kaur Bhatia*, Rajiv Ahluwalia, Stutee Bali Grewal and Dakshina Bisht

Santosh Dental College & Hospital, Pratap Vihar, Ghaziabad (U.P.).

***Corresponding Author: Dr. Navneet Kaur Bhatia**

Santosh Dental College & Hospital, Pratap Vihar, Ghaziabad (U.P.).

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ABSTRACT

The oral environment provides an ideal environment for the colonization of a complex microbiota. When changes occur in the normal oral environment, the balanced flora changes, and imbalance and disease may result. Such changes could be brought about by the introduction of orthodontic appliances. 80 patients undergoing fixed orthodontic treatment were selected following inclusion and exclusion criteria and divided into two equal groups. Study group (SG) of 40 patients undergoing orthodontic treatment were educated by a video of how to maintain proper oral hygiene by using modified bass brushing technique and were instructed to follow the same. Control group (CG) comprising of 40 patients undergoing orthodontic treatment were asked to maintain oral hygiene on its own. Oral examination of the orthodontic patients was performed and modified plaque index was calculated at the start of the treatment, as well as at an interval of 3 months till one year. Two swabs of plaque samples from buccal/ labial and palatal/lingual tooth surfaces were collected by rubbing the tooth surfaces. First swab was cultured on Blood Agar and Mitis Salivarius Bacitracin Agar to determine counts of Streptococcus mutans, and second was cultured on Sabaraud's Dextrose Agar to determine the counts of Candida albicans. 62.5% patients reported with Streptococcus mutans in both the groups at the end of 1st month. At the end of 4th month and 8th month, 62.5% patients in SG and 65% in CG reported with Streptococcus mutans. At the end of 12th month, 65% SG reported Streptococcus mutans as compared to 70% in CG. At the end of 1st month, 57.5% patients in SG reported with Candida albicans as compared to 67.5% patients in CG. At the end of 4th month, 62.5% patients of SG and 72.5% in CG reported with Candida albicans. At the end of 12th month, 72.5% SG patients and 82.5% in CG reported with Candida albicans. The difference was statistically significant. As Streptococcus mutans and Candida albicans are pathogenic microbes leading to dental caries and various other oral diseases, good oral hygiene can play an important role in orthodontic treatment and preventing further dental problems. Modified bass brushing technique is an important tool in maintaining good oral hygiene.

KEYWORDS: Fixed Orthodontic appliances, Modified bass brushing, Habitual brushing, Oral microbes, Streptococcus mutans, Candida albicans.

INTRODUCTION

The oral environment provides an ideal environment for the colonization of a complex microbiota. Many factors, both intrinsic and extrinsic, have an effect on the composition, metabolic activity and pathogenicity of the highly diverse microbial flora in the mouth. In a healthy oral cavity, these microorganisms coexist in a balanced state with their host. But when changes occur in the normal oral environment, the balanced flora changes, and imbalance and disease may result. Such changes could be brought about by the introduction of orthodontic appliances.

Orthodontic brackets and bands have been found to induce specific changes in the buccal environment such as decreased pH and increased plaque accumulation.[1] Malocclusion is the 3rd most common cause of oral health problems, and is associated with a number of complications.[2] Orthodontic treatment can correct these complications or at least prevent them from progressing as orthodontic appliances are effective in producing desired tooth movements for improved functional and aesthetic purposes.[3] It has been widely reported that Fixed orthodontic appliance therapy greatly poses an obstruction in maintaining proper oral hygiene and creates new retentive areas for plaque and debris, which in turn predisposes to increased carriage of microbes and subsequent infection.[4] Thus, there is a link between an increased number of dental plaque in individuals treated with Fixed Orthodontic Appliance and the subsequent development of gingivitis.

Oral debris is loose material (e.g. food particles) that influences the components of microbial flora, in part by providing food sources for the organisms present in plaque.[5] A number of endogenous oral microorganisms found in dental plaque are important in the initiation and progression of dental caries. These microorganisms include mutans streptococci (*Streptococcus mutans*, *Streptococcus sobrinus*), Lactobacillus species, Actinomyces species, nonmutans streptococci and yeast. The microbial traits strongly associated with caries include the ability to produce acid and sustain acid production at low pH levels that result in the demineralization of the calcified structure, the formation and use of extra and intracellular storage polysaccharides that permit microorganisms to continuously produce acid even after dietary carbohydrates have been depleted and lastly the formation of water-insoluble glucans that aid in the accumulation of mutans streptococci in plaque, allowing the substrate to diffuse to a deeper layer of plaque adjacent to the tooth surface. Mutans streptococci possess all of these virulent traits which support their role in the carious process.[6]

Streptococcus mutans are gram-positive cocci shaped bacteria and are a part of normal flora of oral cavity. They become pathogenic only under conditions that lead to frequent and prolonged acidification of the dental plaque.[7] Patients undergoing orthodontic therapy have oral ecological changes that lead to increased numbers of mutans streptococci in saliva and plaque.[8.9]

Candida species is a commensal yeast which colonizes the oropharyngeal region of up to 60% of all healthy immune-competent individuals.[10] The ability of Candida to become a pathogenic microorganism capable of causing infections is attributed to a number of factors.[11] Local oral factors may also influence oral Candida carriage and these mainly include wearing removable dentures, fixed and removable orthodontic appliance, dry mouth, high sugar diet and poor oral hygiene.[12,13]

Patients undergoing orthodontic treatment are more likely to suffer from periodontal diseases as in most of the cases they don't know how to maintain the high levels of oral hygiene which is conducive to excellent orthodontic treatment result.[14] Therefore a current knowledge about oral hygiene with emphasis on practical measure is of great value to the orthodontic patients such as regular tooth brushing. It has been reported that initial caries and gingival inflammation develop only to a minor degree during a 2-year period of orthodontic treatment. This was ascribed to an optimal level of oral hygiene resulting from an intensive educational programme preceding active orthodontic treatment.[15]

Daily brushing and flossing is a preventive care and must be taken by orthodontic patients for good oral health.[16] In these contexts, many types of toothbrushes both manual and powered options have been promoted for orthodontic patients.[17] Presence of dental braces (orthodontic bands, brackets and wires) makes it extremely difficult for a person to brush their teeth effectively. In those situations, where adequate brushing has not been achieved, the person is likely to develop "gingivitis" or there will be formation of "white spot" lesion on the surface of the tooth enamel progressing into a cavity.

The tooth brushing techniques most used in orthodontic patients are: Ramfjord's method, modified Stillman and Bass method.[16] Katz et al (as quoted by Poyato- Ferrera et al[18]) recommended the modification of Bass method combining this technique with the Roll method [modified Bass (Mod-Bass) technique] to ensure complete plaque removal of both coronal surfaces and gingival margins. There are two alternatives to improve the oral hygiene of the patients using tooth brushing i.e. to introduce specific tooth brushing method and to better the performance of normal tooth brushing practices. Most studies have definitively demonstrated that introduction of a specific tooth brushing technique produces a superior increase of oral hygiene that the normal tooth brushing practices perform with improvement.[18]

Aims & Objectives

The aim of the present study was comparative analysis of modified bass brushing technique and habitual brushing on the carriage of Streptococcus mutans and Candida albicans in patients with fixed orthodontic appliances.

The objectives of the study were: -

- To evaluate the prevalence of *Streptococcus mutans*, *Candida albicans* in oral microbial flora in patients with fixed orthodontic appliances [Study Group (SG)].
- To compare the efficacy of modified bass brushing technique with habitual brushing on their counts
- To measure the efficacy of modified bass brushing technique by calculating the Plaque Index at every interval.

MATERIAL AND METHODS

The study was conducted on 80 patients between 12 to 26 years of age (23 males and 57 females) undergoing treatment with fixed orthodontic appliances at Department of Orthodontics and Dentofacial Orthopedics, Santosh Dental College, Ghaziabad. Swabs of plaque samples were taken from these patients and were processed in the lab of Department of Microbiology, Santosh Medical College, Ghaziabad. Institutional ethical clearance and Informed consent was taken.

80 patients undergoing fixed orthodontic treatment were selected and divided into two equal groups. Study Group (SG) consisted of a total number of 40 patients undergoing orthodontic treatment were educated by a video of how to maintain proper oral hygiene by using modified bass brushing technique and were instructed to follow the same. Control group (CG) had an equal

number of 40 patients undergoing orthodontic treatment were asked to maintain oral hygiene on its own.

The following inclusion and exclusion criteria was used.

Inclusion criteria

- Patients undergoing fixed orthodontic therapy of age between 12 to 26 years of age
- Good oral hygiene and periodontal condition at the start of orthodontic treatment
- Non smokers

Exclusion criteria

Patients with

- ill habits like smoking
- gingival and periodontal diseases like gingivitis, periodontitis
- handicapped patients (as they are unable to maintain oral hygiene)
- psychological problems
- does not have any systemic or local disease affecting periodontium and oral mucosa
- patients taking antibiotic and steroid therapy for last 6 months (as it affects oral Candida carriage)

Examination & Sample Collection

1. *Oral examination*

Oral examination of the orthodontic patients was performed and modified plaque index was calculated at the start of the treatment, as well as at an interval of 3 months till one year.

Plaque Index

Plaque Index (PlI) was introduced by Silness and Loe[19] in 1964. It is used on all teeth (28, so wisdom teeth are excluded) or selected teeth (6); No substitution for any missing tooth; Used on all surfaces (4) (Disto-facial, facial, Mesio-facial and lingual). This index measures the thickness of plaque on the gingival one third. The six index teeth are: Maxillary right first molar (16); Maxillary right lateral incisor (12); Maxillary left first bicuspid (24); Mandibular left first molar (36); Mandibular left lateral incisor (32) and Mandibular right first bicuspid (44).

Procedure

A mouth mirror, a dental explorer and air drying of the teeth and gingiva were used. The tooth is dried and examined visually. When no plaque is visible an explorer is used to test the surface. The explorer is passed across the tooth surface in the cervical third and near the entrance to the sulcus. When no plaque adheres to the point of explorer, the area is considered to have a '0' score. When plaque adheres a score of '1' is given. Plaque that is on the surface of calculus deposits and on dental restorations of all types in the cervical third is evaluated and included.

Scoring Criteria

0 - No plaque

1 - A film of plaque adhering to the free gingival margin and adjacent area of the tooth, which cannot be seen with the naked eye. But only by using disclosing solution or by using probe.

2 - Moderate accumulation of deposits within the gingival pocket, on the gingival margin and/ or adjacent tooth surface, which can be seen with the naked eye.

3 - Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

Calculation

1- Individual

2- Population:

PII =Total scores divided by No. of subjects examined

PII = Total scores divided by No. of surfaces examined

Suggested normal scale for Patient evaluation: -

Rating Scores

Excellent 0

Good 0.1 – 0.9

Fair 1.0 – 1.9

Poor 2.0 – 3.0

2. Brushing technique: After oral examination was completed the study group was educated about Modified Bass brushing technique whereas control group was asked to maintain hygiene on its own.

Modified Bass Brushing Technique[20]: This technique combines the vibratory & circular movements of the Bass technique with the sweeping motion of the Roll technique the toothbrush is held at 45^0 to the gingivae. Bristles are gently vibrated by moving the brush handle in a back and forth motion. In a single motion, the bristles are then swept over the sides of the teeth towards their occlusal surfaces. The advantages of this technique are excellent sulcus cleaning, good inter-proximal and gingival cleaning, good gingival stimulation. Disadvantages are moderate dexterity of wrist is required.

3. Sample collection

Both the groups were provided with standard toothpaste and orthodontic brush. Two swabs of plaque samples from buccal/ labial and palatal/lingual tooth surfaces were collected by rubbing the tooth surfaces as shown in Fig 1. First swab was cultured on Blood Agar and Mitis Salivarius Bacitracin Agar to determine counts of *Streptococcus mutans*, and second was cultured on Sabaraud's Dextrose Agar to determine the counts of *Candida* albicans. The patients were evaluated in the afternoon periods after 5-6 hours of brushing at first month then after an interval of every 3 months till twelve months, during fixed orthodontic therapy. Plaque index of the patients was also noted at every interval.

Fig. 1: Sample collection.

Sample processing for Streptococcus mutans

One swab was cultured on 5% sheep blood agar and Mitis salivarius Bacitracin agar (MSB Agar) containing 10% sucrose, 1unit/ml bacitracin, 1ml/1000ml Potassium tellutrite was used. Colony count was performed manually for streptococcal colonies obtained as pure growth by using a magnifying glass. Organism was identified and confirmed by gram staining and standard biochemical tests.

Sample processing for Candida albicans

Gram staining was done to determine morphology of Candida. For species identification of Candida, Germ tube test was performed.[21] The germ tube test provides a simple, reliable and economical procedure for the presumptive identification of *Candida albicans*.

Statistical Analysis

The software used for the statistical analysis were SPSS (statistical package for social sciences) version 21.0 and Epi-info version 3.0. The statistical tests used were: Unpaired or Independent T-test and Chi square test. The p-value was taken significant when less than 0.05 ($p<0.05$) and Confidence interval of 95% was taken.

RESULTS

In the present study, total number of 80 patients between 12 to 26 years of age (23 males and 57 females) undergoing treatment with fixed orthodontic appliances were divided into two equal groups of 40 patients. One group was educated about modified bass brushing technique and other group followed habitual brushing technique. Plaque samples from each of the group were processed and analyzed for Streptococcus mutans and Candida albicans. Comparisons of their counts were made between modified bass brushing technique and habitual brushing and using unpaired t-test.

Prevalence of Streptococcus mutans in modified bass brushing technique group and habitual brushing group

The comparison of prevalence of *Streptococcus mutans* in modified bass brushing technique group and habitual brushing group was compared using the chi-square test. In the modified bass brushing group, 62.5% patients reported with *Streptococcus mutans* in both the groups at the end of 1st month. At the end of 4th month and 8th month, 62.5% patients of modified bass group reported with *Streptococcus mutans* whereas 65% patients reported in the habitual group. At the end of 12th month 65% patients reported *Streptococcus mutans* in modified bass group whereas 70% patients reported in the habitual group. There was a significant difference in the prevalence of *Streptococcus mutans* in between both the groups at 4th, 8th and 12th months (Table 1).

Table 1: Prevalence of Streptococcus mutans in modified bass brushing technique group and habitual brushing group.

Number of patients with *Streptococcus mutans*	1st month	4th month	8th month	12th month
Modified bass brushing technique	25/ 40 (62.5%)	25/40(62.5%)	25/40(62.5%)	26/40(65%)
Habitual brushing	25/40(62.5%)	26/40 (65%)	26/40(65%)	28/40 (70%)
p-value	1.000	0.049*	0.049*	0.041*

Prevalence of Candida albicans in modified bass brushing technique group and habitual brushing group

At the end of 1st month, 57.5% patients in SG reported with Candida albicans as compared to 67.5% patients in CG. At the end of 4th month, 62.5% patients of SG and 72.5% in CG reported with Candida albicans. At the end of 12th month, 72.5% SG patients and 82.5% in CG reported with Candida albicans. There was a significant difference in the prevalence of *Candida albicans* in between both the groups at all intervals as illustrated in Table 2.

Table 2: Prevalence of Candida albicans [N (%)].

Number of patients with Candida albicans	1st month	4th month	8th month	12th month
Modified bass brushing technique	23/ 40 (57.5%)	25/40(62.5%)	26/40(65%)	29/40 (72.5%)
Habitual brushing	27/40 (67.5%)	29/40(72.5%)	32/40 (80%)	33/40 (82.5%)
p-value	0.036*	0.034*	0.033*	0.042*

Prevalence of coexistence of Streptococcus mutans and Candida albicans in modified bass brushing technique group and habitual brushing group

In the modified bass brushing group, 37.5% patients reported with both *Streptococcus mutans* and *Candida albicans* in modified bass group and 45% patients in habitual group at the end of 1st month. At the end of 4th month, 42.5% patients of modified bass group reported with both *Streptococcus mutans* and *Candida albicans* whereas 52.5% patients reported in the habitual group. At the end of 8th month, 45% patients of modified bass group reported with both *Streptococcus mutans* and *Candida albicans* whereas 57.5% patients reported in the habitual group. At the end of 12th month, 52.5% patients of modified bass group reported both whereas 65% patients reported in the habitual group. there was a significant difference in the prevalence of coexistence of *Streptococcus mutans* and *Candida albicans* in between both the groups as illustrated in Table 3.

Table 3: Prevalence of Streptococcus mutans and Candida albicans together [N (%)].

Number of patients with both S. Mutans & Candida albicans	1st month	4th month	8th month	12th month
Modified bass brushing technique	15/ 40 (37.5%)	17/40 (42.5%)	18/40 (45%)	21/40(52.5%)
Habitual brushing	18/40(45%)	21/40(52.5%)	23/40 (57.5%)	26/40 (65%)
p-value	0.049*	0.037*	0.026*	0.025*

Mean values of Streptococcus mutans

The mean *Streptococcus mutans* count was compared between habitual brushing and modified bass brushing technique using unpaired t-test. There was a significant difference between the two groups at 1 month, 4 months, 8 months and 12 months. The mean *Streptococcus mutans* count was significantly more among habitual group in comparison to modified bass brushing technique group at 1 month, 4 months, 8 months and 12 months as illustrated in Table 4.

Table 4: Mean count of Streptococcus mutans.

	Habitual technique		Modified bass technique				
S. mutans count (10^4)	Mean	S.D.	Mean	S.D.	Mean difference	t-test value	p-value
1 month	17.31	2.18	14.74	2.61	2.57	3.380	0.002**
4 months	26.75	3.59	15.57	4.34	11.18	8.877	0.000***
8 months	38.79	5.32	26.85	6.56	11.94	6.322	0.000***
12 months	51.63	7.39	38.91	8.21	12.72	5.150	0.000***

Unpaired or independent t-test
***** Very Highly significant difference (p-value≤0.001)**
**** Highly significant difference (p-value≤0.01)**

Mean Difference in Streptococcus mutans count between intervals

The mean difference in *Streptococcus mutans* count from 1 month to 4, 8 and 12 months, 4 months to 8 and 12 months and from 8 months to 12 months was compared between habitual brushing and modified bass brushing technique using unpaired t-test. There was a significant difference between the two groups count from 1 month to 4, 8 and 12 months, 4 months to 8 and 12 months and from 8 months to 12 months. The mean *Streptococcus mutans* count was significantly more among habitual group in comparison to modified bass brushing technique shown in Table 5.

Table 5: Mean Difference between intervals.

	Habitual technique		Modified bass technique				
	Mean	S.D.	Mean	S.D.	Mean difference	t-test value	p-value
Difference from 1 month to 4 months	9.44	2.44	0.83	0.26	8.61	3.380	0.002**
Difference from 1 month to 8 months	21.48	3.67	12.11	2.67	9.37	8.877	0.000***
Difference from 1 month to 12 months	34.32	5.78	24.17	4.78	10.15	6.322	0.000***
Difference from 4 months to 8 months	12.04	2.23	11.28	2.57	0.76	4.380	0.001***
Difference from 4 months to 12 months	24.88	7.91	23.34	5.79	1.54	7.861	0.000***
Difference from 8 months to 12 months	12.84	1.98	12.06	3.27	0.78	3.340	0.002**

Unpaired or independent t-test
***** Very Highly significant difference (p-value≤0.001)**
**** Highly significant difference (p-value≤0.01)**

Mean values of Candida albicans

The mean *Candida albicans* count was compared between habitual brushing and modified bass brushing technique using unpaired t-test. The mean count was significantly more among habitual group in comparison to modified bass brushing technique group at 1 month, 4 months. However, there was non-significant difference at 8 and 12 months as shown in Table 6.

Table 6: Mean Candida albicans count.

	Habitual technique		Modified bass technique				
c. albicans count in colonies	Mean	S.D.	Mean	S.D.	Mean difference	t-test value	p-value
1 month	14.73	2.38	12.33	2.16	2.40	3.340	0.002**
4 months	19.16	3.79	16.71	4.17	2.45	2.944	0.049*
8 months	22.85	5.82	18.92	6.60	3.93	1.994	0.053#
12 months	25.09	8.29	21.19	9.21	3.90	1.408	0.167#

Unpaired or independent t-test
 # Non- Significant difference (p-value>0.05)
*** Significant difference (p-value≤0.05)**
**** Highly significant difference (p-value≤0.01)**

Mean difference in Candida albicans count

The mean difference in *Candida albicans* count from 1 month to 4, 8 and 12 months, 4 months to 8 and 12 months and from 8 months to 12 months was compared between habitual brushing and modified bass brushing technique using unpaired t-test. There was a significant difference between the two groups count from 1 month to 4 month and 1 to 8 month. The mean *Candida albicans* count was significantly more among habitual group in comparison to modified bass brushing technique during these months. However, no significant difference was found between two groups at 1 month to 12 months, 4 months to 8 and 12 months and from 8 months to 12 months as illustrated in Table 7.

Table 7: Mean difference in Candida albicans count.

	Habitual technique		Modified bass technique				
	Mean	S.D.	Mean	S.D.	Mean difference	t-test value	p-value
Difference from 1 month to 4 months	4.43	1.26	4.38	1.09	0.05	3.340	0.002**
Difference from 1 month to 8 months	8.12	2.67	6.59	1.47	1.53	2.944	0.049*
Difference from 1 month to 12 months	10.36	3.78	8.86	2.7	1.50	1.994	0.053#
Difference from 4 months to 8 months	3.69	0.57	2.21	0.56	1.48	1.408	0.167#
Difference from 4 months to 12 months	5.93	1.79	4.48	1.23	1.45	1.528	0.237#
Difference from 8 months to 12 months	2.24	0.97	2.27	0.86	-0.03	0.879	0.518#

Unpaired or independent t-test
 # Non- Significant difference (p-value>0.05)
*** Significant difference (p-value≤0.05)**

Mean of Plaque Index

The mean of Plaque Index of habitual brushing patients and patients using modified bass brushing technique was compared using unpaired t-test. There was a significant difference between the two groups at 1 month, 4 months, 8 months and 12 months. The mean Plaque Index was significantly more among habitual group in comparison to modified bass brushing technique group at 1 month, 4 months, 8 months and 12 months as illustrated in Table 8.

Table 8: Mean Plaque Index.

	Habitual technique		Modified bass technique				
Plaque index (PI)	Mean	S.D.	Mean	S.D.	Mean difference	t-test value	p-value
1 month	0.69	0.09	0.42	0.07	0.27	10.590	0.000***
4 months	0.89	0.15	0.57	0.13	0.32	6.942	0.000***
8 months	1.04	0.21	0.69	0.24	0.35	4.908	0.000***
12 months	1.43	0.27	1.01	0.21	0.42	5.491	0.000***

Unpaired or independent t-test
***** Very Highly significant difference (p-value≤0.001)**

Mean difference in Plaque Index in different intervals

The mean difference in Plaque Index from 1 month to 4, 8 and 12 months, 4 months to 8 and 12 months and from 8 months to 12 months was compared between habitual brushing and modified bass brushing technique using unpaired t-test. There was a significant difference between the two groups count from 1 month to 8 months, 1 to 12 months, 4 to 12 months and 8 to 12 months. The mean Plaque Index was significantly more among habitual group in comparison to modified bass brushing technique during these months. However, no significant difference was found between two groups at 1 month to 4 months, 4 months to 8 months as illustrated in Table 9.

Table 9: Mean difference in Plaque Index in different intervals.

	Habitual technique		Modified bass technique				
Plaque index	Mean	S.D.	Mean	S.D.	Mean difference	t-test value	p-value
Difference from 1 month to 4 months	0.20	0.05	0.15	0.04	0.05	1.590	0.101#
Difference from 1 month to 8 months	0.35	0.13	0.27	0.11	0.08	3.942	0.044*
Difference from 1 month to 12 months	0.74	0.22	0.59	0.19	0.15	4.908	0.020*
Difference from 4 months to 8 months	0.15	0.04	0.12	0.03	0.03	0.991	0.108#
Difference from 4 months to 12 months	0.54	0.26	0.44	0.17	0.10	4.848	0.010**
Difference from 8 months to 12 months	0.39	0.17	0.32	0.12	0.07	4.042	0.049*

Unpaired or independent t-test
 # Non- Significant difference (p-value>0.05)
*** Significant difference (p-value≤0.05)**
**** Highly Significant difference (p-value≤0.01)**

DISCUSSION

The normal flora of oral cavity is the mixture of organisms regularly found at any anatomical site and it is exceedingly complex and consists of more than 200 species of bacteria. The placement of fixed orthodontic appliances creates a favorable environment for the accumulation of microbiota and food residues, which, in time, may cause caries or exacerbate any pre-existing periodontal disease.[22]

a) Increased Dental plaque in orthodontic patients

Microbial traits strongly associated with caries include the ability to produce acid and to sustain acid production at low pH levels that results in the demineralization of the calcified structure, the formation and use of extra and intracellular storage polysaccharide that permit microorganisms to continuously produce acid even after dietary carbohydrates have been depleted, the formation of water-insoluble glucans that aid in the accumulation of mutans streptococci in plaque and modification of its diffusion characteristics allowing the substrate to diffuse to a deeper layer of plaque adjacent to the tooth surface. The mutans streptococci have long been implicated as significant contributor to the development of human dental caries on booth coronal and root surfaces.[16] Recent studies have reported that demineralization of dental surfaces during treatment can be found in 50 – 75% of all patients with fixed orthodontic appliances.[23,24]

In our study, plaque index (by Silness and Loe[19]) was calculated at the 1st, 4th, 8th and 12th month of the treatment, to measure plaque as well as efficacy of the brushing techniques. In support of our study, Alnezi et al reported that the modified Silness and Loe index may be considered the most valid and discriminatory index to measure plaque in patients with fixed orthodontic appliances.[25]

Our study reported increase in Plaque Index after the insertion of orthodontic appliances in both the groups, the modified bass brushing group and habitual brushing group. However, plaque index increased more in habitual brushing during the course of treatment as compared to modified bass group. Previous studies have suggested that orthodontic appliances result in an increase in plaque, with a consequent increase in bacterial numbers and bacterial byproducts.[26-28] Contrary to our study, Davies et al 1991, reported that patients who received orthodontic treatment had the greater plaque reduction due to behavioral factors.[29]

b) Role of *Streptococcus mutans* in the oral cavity

Streptococcus mutans is one of the bacteria most frequently implicated in decalcification of enamel.[30] Different agar media used to study streptococcus mutans are mitis salivarius with bacitracin (MSB), tripticase soy with sucrose and bacitracin (TYSCB) and mitis salivarius with bacitracin and kanyamycin (MSKB).

Different chair side cultural tests can also be used for enumeration of mutans streptococci.[31] In our study MSB was used to detect *Streptococcus mutans*.

Streptococcus mutans was isolated in 50-80% of orthodontic patients as a common cause of decalcification due to accumulation of cariogenic plaque around the brackets progressing into carious lesions in such patients. In the present study, 67% of patients with fixed orthodontic appliances using modified bass brushing technique were positive for *Streptococcus mutans* and 77% of patients with fixed orthodontic appliances using habitual brushing were positive.

Our study reported significant increase in levels of *Streptococcus mutans* during the initial months of treatment which is supported by Corbett et al who stated that regardless of dental caries status, banded patients had significantly greater buccolingual *Streptococcus mutans* plaque populations.[32] Scheie also reported that after 3 months of extensive banding, the *Streptococcus mutans* proportions surpassed pretreatment levels in saliva and on banded teeth, whereas unbanded surfaces only showed a slight increase.[33] Many studies[34-36] had reported a significant increase in the percentage of *Streptococcus mutans* in patients with orthodontic appliances similar to the results of our study. In contrast, a study by Mota et al which reported that no significant modification in the number of *Streptococcus mutans* in saliva was observed after placement of the fixed orthodontic appliances in the beginning of the treatment.[37]

c) Role of Candida albicans in the oral cavity

Candida species are known as the most common human oral micro flora, which colonizes in the oral cavity of up to 60% of all healthy individuals with average, 300 to 500 colony forming units per millilitre of saliva. Several local oral factors such as wearing removable complete dentures, fixed and removable orthodontic appliances, dry mouth, high-sugar diet, and poor oral hygiene can increase the oral *Candida* carriage changed to pathogenic form and caused *Candida* -associated buccal lesions.

Hibino *et al.* reported *Candida albicans* as an opportunistic pathogen, is commonly isolated from the mouth of orthodontic patients with removable appliances, formed *Candida* biofilm.[38] Also, *Candida albicans* may also be isolated from the mouth of patients using orthodontic devices.[39,40] In the present study 77% of patients with fixed orthodontic appliances using modified bass brushing technique showed colonization with Candida and 87% of patients with fixed orthodontic appliances using habitual brushing showed colonization with Candida. It has been shown that oral appliances increase the oral Candida carriage rate, as in a long term follow-up study by Arslan et al[41], the Candida counts in patients significantly increased from the 1st month to 6 month and from 1st month to 12 month similar to our study in which the Candida counts in patients significantly increased from the 1st month to 4 month and from 1st month to 8 month in both the groups. Sharma et al also reported the increase in Candida albicans during the course of orthodontic treatment.[35] Patients who have low levels of vitamin B12 and red cell folate may be considered at risk of Candida colonization after the insertion of fixed orthodontic appliances.

In our study, swab was taken from the tooth surfaces to assess candida colonies. Another study used more than one technique for isolation of Candida which were imprint culture, oral rinse and pooled plaque technique. In that study the overall Candida prevalence rates obtained by using the oral rinse technique did not show an increase in Candida density after fixed orthodontic appliance insertion, in contrary to the imprint technique which showed a significant increase.[12]

d) Effects of brushing technique on the carriage of oral microbes in fixed orthodontic patients

Lundstorm[42] reported that following an introductory period including intense dental health education a high level of oral hygiene can be established. Advice on hygiene, given to the patients undergoing appliance therapy, has three objectives: to prevent enamel decalcification, to reduce gingival inflammation and to reduce appliance breakage.[43] The presence of a fixed appliance greatly complicates tooth cleansing; the arch wires and the attachments act as a barrier to the toothbrush bristles, whilst at the same time tending to accumulate plaque and debris.[42] There is substantial evidence that manual toothbrushes are effective in removing bacterial plaque and preventing gingivitis.

Williams et al reported that more patients subjectively preferred the orthodontic brush than the standard brush but there is small but significant superiority of the orthodontic brush in respect of plaque removal on the buccal surfaces mainly confined to the anterior teeth.[44]

Tooth brushing techniques

The normal tooth brushing practices adequately performed by anyone could be sufficient to control bacterial plaque but Bass method and the Roll method are being the most commonly recommended techniques in dental practice. The Bass technique is superior to the Roll method in cleaning the tooth tissue adjacent in the gingival tissue, the gingival margins and the sulcus. Katz et al (as quoted by Poyato-Ferrera et al[18]), recommended the modification of Bass method combining this technique with the Roll method (modified Bass method (Mod – Bass) technique to ensure the complete removal of both coronal and gingival margins.

Modified bass brushing technique

Modified Bass Brushing Technique - This technique combines the vibratory and circular movements of the Bass technique with the sweeping motion of the Roll technique the toothbrush is held at 45° to the gingivae. Bristles are gently vibrated by moving the brush handle

in a back and forth motion. In a single motion, the bristles are then swept over the sides of the teeth towards their occlusal surfaces.[20]

Ferrera et al concluded that modified bass brushing technique is superior to normal tooth brushing practices in supra-gingival plaque removal ($p < 0.05$), introduction of this technique represents an important improvement in oral hygiene which is similar to our results which concluded that plaque index as well bacterial counts are less in modified bass brushing technique group.[45] Nas Bass technique can be effective in progressive and significant reduction of Plaque Index ($p < 0.01$) in patients with fixed orthodontic appliances as compared to other methods like Ramfjord's method and Modified Stillman technique.[45] Huber et al reported in a study that monthly oral hygiene instruction was effective in significantly reducing the amount of visible plaque and gingival inflammation.[46] In our study, patients were regularly educated about how to maintain oral hygiene as suggested by previous studies. Wainwright suggested higher grades of evidence of effectiveness of tooth brushing techniques are required to inform professional bodies that develop guidelines on tooth brushing.[47] It was also observed by Costa et al[17] and Sharma et al[35] that *S mutans* counts were markedly decreased ($p < 0.05$) in the electric and ultrasonic groups in orthodontic patients as compared to manual while Arici et al[48] suggested interdental brushes best for maintaining oral hygiene.

CONCLUSION

As *Streptococcus mutans* and *Candida albicans* are pathogenic microbes and leads to dental caries and various other oral diseases, good oral hygiene plays an important role in orthodontic treatment and preventing further dental problems. Modified bass brushing technique is an important tool in maintaining good oral hygiene.

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