Effects of fixed orthodontic appliance on the microflora of dental plaque

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ABSTRACT

This study was undertaken to elucidate the effect of fixed orthodontic appliance on the microbial composition of dental plaque and to detect any difference between the isolated microflora from different teeth before and during treatment. Twenty subjects (15 females and 5 males) between (12) and (23) years of age participated in this study. From each subject, four swabs were collected; two in the anterior region (upper and lower central incisors) and two in the posterior region (upper and lower first molars). Bacterial examinations were performed before the beginning of the treatment and (4–6) weeks after placement of orthodontic appliances; so (160) swabs were collected. The composition of dental plaque determined was under light microscope. The statistical analysis was performed and the results showed a variety of microorganisms from supra-gingival plaque samples in pre- and during treatment fixed orthodontic patient. Changes during treatment consisted of an increase in the Peptpcocci, Klebsiella, α- Streptococci, Pseudomonas and *Escherichia coli* in certain locations. Conversely, a decrease in the Peptostreptococci, Bacteroids, Veillonella, Staphylococci, *Streptococcus mutans*, Klebsella, Candida, Lactobacilli and Propeniobacterium in different locations; whereas other microorganisms and some of the above-mentioned microorganisms in certain locations showed insignificant differences.

The comparison of microflora between different teeth indicated that the molars presented a significantly more microorganisms than incisors did specially in pre-treatment, whereas the upper incisor in both pre- and during treatment presented more microorganisms than lower incisor; while the lower molar exhibited more microorganisms than the upper molar particularly during treatment.

Key Words: Microbial flora, plaque, fixed appliance treatment.

الخلاصة

وضعت هذه الدراسة لتوضيح تأثير جهاز التقويم الثابت على الجراثيم الفموية الموجودة في الصفيحة الجرثومية ولتعيين أي اختلاف بين الجراثيم المعزولة من الأسنان المختلفة قبل وخلال العلاج. شارك عشرون شخصاً في هذه الدراسة (١٥ أنثى و ٥ ذكور) تتراوح أعمارهم ما بين (١٢) و (٢٣) سنة. من كل شخص تم جمع (٤) مسحات، اثنان في المنطقة الأمامية (القاطع الأوسط العلوي والسفلي) واثنان في المنطقة الخلفية (الطاحن السادس العلوي والسفلي). وقد أُنجز الفحص الجرثومي قبل العلاج وبعد مرور (٤-٦) أسابيع من وضع جهاز التقويم الثابت، ولذلك تم جمع (١٦٠) مسحة من الصفيحة الجرثومية الخارجية. تم تعيين مكونات الصفيحة الجرثومية السنية تحت الميكروسكوب الضوئي.

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أجري التحليل الإحصائي وأظهرت مسحات الصفيحة الجرثومية الخارجية قبل وأثناء علاج التقويم الثابت أنواع مختلفة من الجراثيم. تضمنت التغييرات أثناء فترة العلاج زيادة في البيبتوكوكاي، المكورات العقدية ألفا، الزائفة والأشريكية القولونية في مناطق معينة. وعلى العكس، كان هناك نقصان في البيبتوستربتوكوكاي، العصوائية، فيلونيلا، المكورات العنقودية، المكورات العقدية، الكلسية، المبيضات، المليفات و بروبيوبكتريم في مناطق مختلفة. بينما كانت هناك أنواع أخرى من الأحياء المجهرية لم تظهر أي فرق معنوي ما بين قبل وأثناء فترة العلاج في مناطق معينة. كما أشارت مقارنة الجراثيم بين الأسنان المختلفة إلى أن نسبة وجود الجراثيم في السن الخلفي أكثر من السن الأمامي وخصوصاً قبل العلاج. في حين أظهر السن الأمامي العلوي قبل وأثناء العلاج وجود جراثيم أكثر من السن الأمامي السفلي. بينما وُجِدَ بأن نسبة الجراثيم في السن الخلفي السفلي كانت أكثر من السن الخلفي العلوي وخصوصاً خلال العلاج.

INTRODUCTION

The microbial flora in oral cavity existed in a symbiotic balanced state among its members and the host. When the oral environment can disrupt this balanced state and cause a change in the quantitative flora of the microorganisms [1].

Several researchers [2-4] have attempted to quantify the distribution of organisms present in the plaque of orthodontic patient supra-gingivally. Others [5-7] determined the nature and type of microorganisms that may reside in gingival crevice adjacent to orthodontic appliances.

The introduction of fixed orthodontic appliances into the mouth increases the number of areas for potential plaque retention. Such areas as elastomerics and metals (arch wires, bands and brackets) and exposure of adhesive resin margins [8], and thus the possibility with changes in the microbial environment [9, 10].

Many authors [3, 11-17] have shown elevated Streptococcus colonization in the oral flora for patient undergoing fixed orthodontic treatment. The results of Huser [10] showed that changes consisted of increase in the percentage of Spirochetes, motile rods, filaments ands fusiforms. Conversely, a decrease in cocci was noted; while Diamonti-Kipioti *et al.* [9], in their longitudinal study, found no significant variations in plaque or gingival indices after initiation of orthodontic treatment. Other studies indicated no long-term effect on the supporting tissues [19]. While Feliu [20] found that the patient who had received orthodontic treatment displayed superior oral hygiene to those dental patients who had not received orthodontic treatment. This is possibly due to the emphasis that the orthodontist places on good oral hygiene during treatment. Orthodontic patients make frequent visits to their orthodontists over a period of several months, so there is ample opportunity for them to learn techniques that promote good oral hygiene.

Al-Sheakli [21], in her cross sectional study of the effect of the removable and fixed orthodontic appliance on the microflora of dental plaque, found that the supra-gingival plaque samples from pre- and during treatment orthodontic patients showed a variety of microorganisms; also found that both facultative anaerobic and aerobic microorganisms increased during treatment both on molars and incisors. In addition, the molars presented significantly more microorganisms than incisors did.

AIMS OF THE STUDY

① To investigate some of the aerobic and anaerobic microflora associated with dental patients undergoing fixed orthodontic treatment before and during the treatment.

② To determine the effect of fixed orthodontic treatment on specific isolated oral microflora.
③ To detect any difference between the isolated microflora from different teeth.

MATERIALS AND METHODS

The Sample

The sample consisted of twenty subjects (15 females and 5 males) ranging in age from (12) to (23) years attending the Department of Orthodontics, College of Dentistry, Mosul University. The patients met the following criteria:

All were in good medical health. No previous orthodontic treatment. No extraction of one or both permanent first molars. No unerupted or extracted permanent central incisors. No history of treatment with antibiotic or antimicrobial agents for the past two weeks. Any medication used from first sample collected to second sample collection. The type of malocclusion was not considered in the selection criteria.

A total of (40) permanent upper and lower first molars and (40) permanent upper and lower central incisors were included in this study. Each patient was instructed in the Bass brushing technique. No other professional prophylaxis care was given during the study [10].

Swabbing Method

Four sites were selected in each subject: Two sites in the anterior region (the labial surface of the upper right and lower left permanent central incisor), and two in the posterior site (the buccal surface of the upper left and lower right permanent first molar).

From each tooth surface two swabs were taken with sterile disposable swabs, one for aerobic and the other for anaerobic bacteriological study [10, 11, 22]. All these swabs were cultured aerobically and anaerobically. Swabs were taken from supra-gingival plaque twice, one before appliance insertion and the other time (4) to (6) weeks later after the placement of the orthodontic appliance [11, 23-26].

On the day of swab taking, the patients were forbidden from brushing their teeth. Only rinsing with tap water was allowed. The swab was performed between (9) and (11) a.m. being the least one hour after the patient last ate [10, 27].

Bacteriological Culture, Isolation and Identification of microorganisms

The swabs were immediately inserted in nutrient broth (for aerobic cultures) and the broth was incubated for (24) hours at (37)°C [28], and in thioglycollate broth (for anaerobic cultures) and incubated for (48–72) hours at (37)°C [29]. Then, these swabs were smeared on glass slides for preparing direct smear and stained with Gram's stain to detect the types of microorganisms that were present [30].

The nutrient broths that showed turbidity after (24) hours were cultured on blood agar (to detect the hæmolysis and non-hæmolysis bacteria). Chocolate and MacConkey's agars for aerobic isolation, on Sabouraud dextrose agar to detect and isolate Candida [31, 32]. Whereas the broths that showed no turbidity were excluded because no microbial growth appeared. So the above plates containing the seeded media were incubated in the incubator aerobically at (37)°C for (24–48) hours [28].

The turbidity of thioglycollate broths appears clearly after (48–72) hours and only turbid broths were cultured on blood agar. Ragosa SL media, Schaedler agar and *Mitis salivarius* and incubated anaerobically.

For anaerobic incubation, the anaerobic jar was supplied with Gas pak (oxoid, England), a H_2 and CO_2 generator envelope, which is activated simply by adding (10) ml for distilled water. Then the jar was closed properly and incubated at (37)°C for (48–72) hours [29, 33].

After the time of culturing, the aerobic and anaerobic culture plates were examined and checked under light microscope. The morphology of different types of colonies were smeared to study the isolated type and then sub-cultured to get isolated colonies and made biochemical test on each microorganism which include [28, 33].

Biochemical Tests and Reagents: Include the following:
1) Gram's stain; 2) Sugars; 3) Serum; 4) Normal saline; 5) Human plasma; 6) H_2O_2 (3%); 7) Tetramethyl–p–phenylene diamine hydrochloride (1%) solution; 8) Kovac's reagent (oxoid, England); 9) Simmon citrate (oxoid, England); 10) Distilled water; 11) Triple sugar iron (Difco, USA); 12) Urease test (Difco, USA).

Statistical Analysis

Descriptive statistics including frequency tables and percentages were calculated for all types of microorganisms to the various test variables (sample location including upper and lower in the anterior and posterior sites, and time of sampling).

The pooled Z-test was employed to show any statistically significant association between the tested variables for each microorganism. If the value of Z is less than (1.96) it was regarded as statistically insignificant, whereas the value of Z is more than (1.96) it was considered as significant.

RESULTS

Tables (1), (2) and (3) showed the occurrence of microorganisms in the swabs according to: Time of sampling, location and both the location and time respectively.

Table (1): Occurrence of microorganisms in the swabs according to time of sampling

Occurrence	Pre - treatment (n = 80)	During Treatment (n = 80)	Total (n =160)
Positive	75 (93.8 %)	47 (58.8 %)	122 (76.3 %)
Negative	5 (6.3 %)	33 (41.3 %)	38 (23.8 %)

Table (2): Occurrence of microorganisms in the swabs according to location of sampling

Location	Occurrence	Upper (n =40)	Lower (n =40)	Total (n =80)
Molar	Positive	32 (80 %)	31 (77.5 %)	63 (78.8%)
	Negative	8 (20 %)	9 (22.5%)	17 (21.3 %)
Incisors	Positive	31 (77.5 %)	28 (70 %)	60 (75 %)
	Negative	9 (22.5%)	12 (30 %)	20 (25 %)

Table (3): Occurrence of microorganisms in the swabs according to location and time of sampling

Time of Sampling	Occurrence	Upper			Lower		
		Molar (n=20)	Incisor (n=20)	Total (n=40)	Molar (n=20)	Incisor (n=20)	Total (n=40)
Pre Treatment	Positive	19 (95%)	19 (95%)	38 (95 %)	19 (95%)	18 (90%)	37 (92.5%)
	Negative	1 (5%)	1 (5%)	2 (5 %)	1(5%)	2 (10%)	3 (7.5%)
During Treatment	Positive	13 (65%)	12 (60%)	25 (62.5%)	12 (60%)	10 (50%)	22 (55%)
	Negative	7(35%)	8 (40 %)	15 (73.5%)	8 (40 %)	10 (50%)	18 (45%)

Table (4) revealed the prevalence of the different types of microorganisms before and during orthodontic treatment. It was found the most predominant aerobic bacteria was Staphylococcus and Streptococcus, while the most predominant anaerobic bacteria was Peptostreptococcus and Veillonella in pre- and during treatment.

Table (4): Prevalence of the different types of microorganisms before and during orthodontic treatment

Bacteria	Pre (n=80)	During (n=80)	Total (n=160)
Facultative Anaerobic	69 (86.3 %)	43 (54.1 %)	112 (70.2 %)
G + Cocci	42 (52.5 %)	35 (43.8 %)	77 (48.1 %)
Streptococcus mutans	10 (12.5 %)	2 (2.5 %)	12 (15 %)
α- Streptococcus	4 (5 %)	14 (17.5 %)	18 (22.5 %)
β- Streptococcus	2 (2.5 %)	0 (0 %)	2 (2.5 %)
Staphylococcus	26 (32.5 %)	15 (18.8 %)	41 (25.7 %)
G + Bacilli (Lactobacilli)	5 (6.3 %)	0 (0 %)	5 (2.5 %)
G – Cocci (Neisseria)	4 (5 %)	0 (0 %)	4 (2.5 %)
G – Bacilli	18 (22.5 %)	22 (27.5 %)	40 (25 %)
E. Coli	2 (2.5%)	2 (2.5 %)	4 (2.5 %)
Pseudomonas	6 (7.5 %)	10 (12.5 %)	16 (10 %)
Klebsiella	10 (12.5%)	5 (6.3 %)	15 (9.4 %)
Anaerobes	74 (92.5 %)	27 (33.8 %)	101 (63.1 %)
G + Cocci	34 (42.5 %)	20 (25 %)	54 (33.8 %)
Peptostreptococci	33 (41.3%)	13 ((16.3 %)	46 (28.8 %)
Peptococci	0 (0%)	8 (10 %)	8 (5 %)
G + Bacilli (Propenobacterium)	3 (3.8 %)	0 (0%)	3 (1.9 %)
G – Cocci (Veillonilla)	20 (25%)	11 (13.8%)	31 (19.4 %)
G – bacilli (Bacteroid)	16 (20%)	7 (8.8 %)	23 (14.4 %)
Candida	6 (7.5%)	0 (0%)	6 (3.8 %)

* Pre =Pretreatment , During = During treatment

The comparison of the different types of microorganisms between pre- and during treatment in different locations illustrated in table (5). The only significant increase of the anaerobic microorganisms during treatment was Gram positive cocci (Peptococci) in all locations, while there was a decrease in Peptostreptococci, Bacteroid and Veillonella during treatment in all regions. In addition, there was a decrease in Gram positive bacilli and Gram positive cocci in upper anterior region and Gram positive bacilli in lower posterior region.

From the facultative anaerobic bacteria, the only significant increase during treatment were Streptococcus and Klebsiella in the upper incisor and Pseudomonas in the lower incisor, and Streptococcus and *Escherichia coli* in the lower molar, whereas there were a significant decrease in Staphylococcus, *Streptococcus mutans* in all locations and Klebsiella in upper and lower molar and lower incisor and *Escherichia coli* in upper molar and lower incisor and Candida in lower molar. Other aerobic and anaerobic microorganisms showed insignificant differences between pre- and during treatment.

Table (5): Comparison of the different types of microorganisms according to location and time of sampling between pre and during treatment in different location

Bacteria	Upper						Lower					
	Molar			Incisor			Molar			Incisor		
	P	D	Sig	P	D	Sig	P	D	Sig	P	D	Sig
Facultative Anaerobic	20	10	S	14	12	NS	18	12	S	17	8	S
G + Cocci	13	8	S	10	10	NS	9	5	S	4	8	S
Streptococcus mutans	2	2	NS	2	0	S	4	0	S	2	0	S
a- Streptococcus	1	2	NS	2	7	S	0	3	S	1	2	NS
b- Streptococcus	1	0	S	0	0	NS	0	0	NS	1	0	S
Staphylococcus	9	5	S	6	7	NS	5	3	S	6	5	NS
G +Bacilli (Lactobacilli)	0	0	NS	2	0	S	2	0	S	1	0	S
G – Cocci (Neisseria)	1	0	S	2	0	S	0	0	NS	1	0	S
G – Bacilli	4	0	S	3	5	NS	6	8	NS	5	7	NS
E. Coli	1	0	S	0	0	NS	0	2	S	1	0	S
Pseudomonas	0	0	NS	2	2	NS	4	5	NS	0	3	S
Klebsiella	3	0	S	1	3	S	2	0	S	4	2	S
Anaerobes	18	8	S	18	5	S	19	10	S	17	3	S
G + Cocci	9	8	NS	7	2	S	9	7	NS	9	3	NS
Pepto strepto.	9	5	S	6	2	S	9	3	S	9	3	S
Pepto cocci	0	2	S	0	2	S	0	2	S	0	2	S
G+Bacilli (Propenobacterium)	0	0	NS	2	0	S	1	0	S	0	0	NS
G – Cocci (Veillonilla)	6	2	S	4	5	NS	5	2	S	5	2	S
G – bacilli (Bacteroid)	3	3	NS	4	2	S	5	2	S	4	0	S
Candida	2	3	NS	0	0	NS	4	0	S	0	0	NS

* P =Pretreatment , D = During treatment .

* n = 20 for each group .

* S =significant if the value of Z is greater than 1.69 , N.S = not significant if the value of Z is less than 1.69 .

The comparison of different types of microorganisms between molar and incisor sites in the upper and lower arches before and during treatment were shown in table (6), while the comparison of different types of microorganisms between upper and lower arches in the posterior and anterior sites before and during treatment were illustrated in table (7).

Table (6): Comparison of the different types of microorganisms between molar and incisor in the upper and lower arch before and during orthodontic treatment

Bacteria	Pre-treatment						During Treatment					
	Upper			Lower			Upper			Lower		
	M	I	Sig	M	I	Sig	M	I	Sig	M	I	Sig
Facultative Anaerobic	20	14	S	18	10	S	10	12	NS	12	8	S
G + Cocci	13	10	NS	9	4	S	8	10	NS	8	8	NS
Streptococcus mutans	2	2	NS	4	2	S	2	0	S	0	0	NS
α- Streptococcus	1	2	S	0	1	S	2	7	S	3	2	S
β- Streptococcus	1	0	S	0	1	S	0	0	NS	0	0	NS
Staphylococcus	9	6	S	5	6	NS	5	7	NS	3	5	S
G + Bacilli (Lactobacilli)	0	2	S	2	1	S	0	0	NS	0	0	NS
G – Cocci (Neisseria)	1	2	NS	0	1	S	0	0	NS	0	0	NS
G – Bacilli	4	3	NS	6	5	NS	0	5	S	8	7	NS
E. Coli	1	0	S	0	1	S	0	0	NS	2	0	S
Pseudomonas	0	2	S	4	0	S	0	2	S	5	3	S
Klebsiella	3	1	S	2	4	S	0	3	S	0	2	S
Anaerobes	18	18	NS	19	17	NS	8	5	S	10	3	S
G + Cocci	9	7	S	9	9	NS	8	2	S	7	3	S
Peptostreptococci	9	6	S	9	9	NS	5	2	S	3	3	NS
Peptococci	0	0	NS	0	0	NS	2	2	NS	2	2	NS
G + Bacilli (Propenobacterium)	0	2	S	1	0	S	0	0	NS	0	0	NS
G – Cocci (Veillonilla)	6	4	NS	5	5	NS	2	5	S	2	2	NS
G – bacilli (Bacteroid)	3	4	NS	5	4	NS	3	2	NS	2	0	S
Candida	2	0	S	4	0	S	3	0	S	0	0	NS

* M = molar , I = incisor .
* n = 20 for each group .
* S =significant if the value of Z is greater than 1.69 , N.S = not significant if the value of Z is less than 1.69 .

Table (7): Comparison of the different types of microorganisms between upper and lower arch in the molar and incisor sites before and during treatment

Bacteria	Pre-treatment						During Treatment					
	Molar			Incisor			Molar			Incisor		
	U	L	Sig	U	L	Sig	U	L	Sig	U	L	Sig
Facultative Anaerobic	20	18	NS	14	12	NS	10	12	NS	12	8	S
G + Cocci	13	9	S	10	4	S	8	8	NS	10	8	NS
Streptococcus mutance	2	4	S	2	2	NS	2	0	S	0	0	NS
a- streptococcus	1	0	S	2	1	S	2	3	NS	7	2	S
b- streptococcus	1	0	S	0	1	S	0	0	NS	0	0	NS
staphalyococcus	9	5	S	6	6	NS	5	3	NS	6	5	NS
G + Bacilli(lactobacilli)	0	2	S	2	1	S	0	0	NS	0	0	NS
G – Cocci(Neisseria)	1	0	S	2	1	NS	0	0	NS	0	0	NS
G – Bacilli	4	6	NS	3	5	NS	0	8	S	5	7	NS
E. Coli	1	0	S	0	1	S	0	2	NS	0	0	NS
Pseudomonas	0	4	S	2	0	S	0	3	S	2	3	NS
Klebsiella	3	2	NS	1	4	S	0	0	NS	3	2	NS
Anaerobes	18	19	NS	18	17	NS	8	10	NS	5	3	NS
G + Cocci	9	9	NS	7	9	NS	8	7	NS	2	3	NS
Peptostreptococci	9	9	NS	6	9	S	5	3	NS	2	3	NS
Peptococci	0	0	NS	0	0	NS	2	2	NS	2	2	NS
G + Bacilli (Propenobacterium)	0	1	S	2	0	S	0	0	NS	0	0	NS
G – Cocci (veillonilla)	6	5	NS	4	5	NS	2	2	NS	5	2	S
G – bacilli(bacteroid)	3	5	S	4	4	NS	3	2	NS	2	0	S
Candida	2	4	S	0	0	NS	3	0	NS	0	0	NS

* U = upper , L = lower .
* n = 20 for each group .
* S=significant if the value of Z is greater than 1.69 , N.S = not significant if the value of Z is less than 1.69 .

DISCUSSION

Since the low percentage of the males from the total number of patients, therefore findings are presented the sexes combined. Also many studies [1, 34, 35] on orthodontic patients not consider the sex relation to microorganism. While others [35, 36] found no relation between sex and microorganisms in orthodontic patients.

The sample in this study was including permanent dentition only to reduce the age variation. Also, in this period, the quality of microorganisms was affected than the quantity due to present gingival crevice and anatomical feature of permanent teeth as presence of grooves and cusps [37].

Comparison of Microorganisms According to Time

The comparison of different types of microorganisms between pre- and during treatment in general was shown in table (4) and in different locations was illustrated in table (5).

In early plaque formation, after placement of fixed appliance, there was increase in the number of α- Streptococcus in the upper incisor and lower molar. This is in agreement with the findings of Al-Sheakli [21] and Sinclair *et al.* [39]; and other researchers [12, 18] have suggested that an increase in streptococcal flora can lead to a higher incidence of caries. The other types of facultative anaerobic microorganisms are Gram negative bacilli. Also show increase in treatment in certain locations especially Pseudomonas in the lower incisor and Klebsiella in the upper incisor. This is in accordance with Al-Sheakli [21]. This study also showed increase in *Escherichia coli* in the lower molar.

From anaerobic bacteria, the only significant increase during treatment was Gram positive cocci (Peptococci) in all locations. Other authors [1, 10, 21] also showed significant increase in Gram positive cocci especially Peptostreptococci than Peptococci.

On the other hand, there was a decrease in Staphylococcus, *Streptococcus mutans*, Peptostreptococcus, Bacteroid and Veillonella during treatment in all locations. In addition, there was a decrease in Gram positive bacilli and cocci in upper incisor, *Escherichia coli* and Klebsiella in lower incisor, Gram positive bacilli, Klebsiella and Candida in lower molar, and *Escherichia coli* in upper molar. This may be due to the relatively good oral hygiene [20], particularly most of the sample studied was females who had more care for general oral hygiene causes less accumulation of microbial plaque. This is in agreement with Bloom & Brown [2] and Schuster [40].

Other aerobic and anaerobic microorganisms showed insignificant differences between pre- and during treatment in certain locations. This may be due to either the need for more time to propagate in plaque and the plaque not mature enough to get more significant differences in these microorganisms, or these types of microorganisms associated caries lesion initiation. This agrees with Bloom & Brown [2], Vanpalestein [41], Diamonti-Kipiotti [9], while disagrees with Owen [42], Nolte [43], Huser *et al.* [10], Patti & Arca [44].

Comparison of Microorganisms According to Location of Swabbing

The comparison of the molar and incisor in the upper and lower arches as shown in table (6) in pre- treatment indicated a significant increase in the number of Staphylococcus, Klebsiella, β- Streptococcus, *Escherichia coli*, Candida and Peptostreptococcus in the upper molars than in upper incisors. In contrast, there was a significant increase in the Streptococcus, Pseudomonas, Lactobacilli, Neisseria, Gram positive bacilli and Gram positive cocci in the upper incisor.

While in the lower arch, there was increase in the *Streptococcus mutans* Pseudomonas, Lactobacilli, Candida and Gram positive bacilli (Propeniobacterium) in the molar, whereas a significant increase in the Klebsiella, α- Streptococcus, *Escherichia coli*, Neisseria, β- Streptococcus in the lower molar than lower incisor.

While after placement of the fixed appliance, there was a significant increase in the *Streptococcus mutans*, Candida and Peptostreptococcus in the upper molars. On the other hand, the upper incisor exhibited more levels of Streptococcus, Klebsiella, Pseudomonas and Veillonella than the upper molars; whereas in the lower arch the lower molar showed the elevated level of Streptococcus, Pseudomonas, *Escherichia coli* and Bacteroid in the molar, while the lower incisor exhibited a large number of Staphylococcus and Klebsiella.

In general, from the above mentioned, it was noted that the molar presented more microorganisms than incisors did in both upper and lower arches and in both pre- and during treatment especially in the lower arch in pre- treatment, and this agrees with many researchers because:

1. The molar surface is larger than incisor.
2. The presence of grooves, fissures and cusps makes a good area for accumulation of microorganisms.
3. The position of molars in the anterior region makes a good environment and considered as an ideal microbial incubation for all types of microorganisms [10, 43, 45-48], in addition to the personal care for brushing posterior teeth is less adequate than for the anterior teeth [10, 30, 42, 49-51].

While after placement of fixed appliance, the significant difference between molar and incisor was less than that in pre- treatment. This may attributed to direct bonding increasing some species of Gram positive facultative anaerobic cocci types. This agrees with other studies [11, 17, 44, 52, 53].

The comparison of different types of microorganisms between the upper and lower molar as illustrated in table (7) showed a significant increase in the number of microorganisms in the lower molar (including *Streptococcus mutans*, Lactobacilli, Pseudomonas, Propeniobacterium, Bacteroid and Candida) than the upper molar in pre- treatment.

While the comparison of the upper and lower incisor indicated that in pre-treatment revealed a significant increase for α-Streptococcus, Lactobacilli, Pseudomonas, Gram positive cocci, Propeniobacterium in the upper incisor, whereas there is a significant increase in the lower incisor for β- Streptococcus, *Escherichia coli*, Klebsiella and Peptostreptococcus.

While during treatment, generally, there is no significant difference between upper and lower molars except for the *Streptococcus mutans* in the upper molar and Pseudomonas in the lower molars; whereas in the incisor region, there was a significant increase in the α- Streptococcus, Veillonella and Bacteroid in the upper incisor only when compared with the lower incisor.

In this study as in table (7) generally found the upper incisor in both pre- and during treatment presented more microorganisms than lower incisor did. In contrast, the lower molar exhibited more microorganisms than the upper molar did. This may be attributed to larger surface area of both upper incisor and lower molar than that controversy.

CONCLUSIONS

1) Supra-gingival plaque samples from pre- and during treatment orthodontic patients showed a variety of microorganisms; namely, both facultative an-aerobic and anaerobic, Gram positive and negative cocci and bacilli together with Candida.
2) In early plaque formation, after placement of appliance, changes consisted of an increase in the percentage of certain types of microorganisms. From these groups, the α-Streptococcus, Peptococci and Pseudomonas were most significantly affected types. Conversely, there was a decrease in the number of other microorganisms; of those, Peptostreptococcus then Staphylococcus and Bacteroid were found to be the most affected types, whereas other microorganisms showed insignificant differences between pre- and during treatment.
3) Molars presented significant more microorganisms than incisors did especially in pre- treatment.
4) Both pre- and during treatment, molars presented significantly more microorganisms than incisors did especially pre- treatment.

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