

The Effect of Upper Removable Orthodontic Appliances on Oral Candidal Mucosal Carriage

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ABSTRACT

Background: Treatment of malocclusions using removable orthodontic appliances may prepare new stagnant areas susceptible for colonization and retention of *Candida* species, therefore; the aim of this study was to investigate the effect of upper removable orthodontic appliances on the mucosal *Candidal* count in group of apparently healthy orthodontic patients.

Materials and Methods: Patients enrolled in this study were children aged 8-12 years having upper removable orthodontic appliances. *Candidal* counts at six mucosal sites were estimated using Imprint Culture method before, 14 and 28 days during orthodontic therapy.

Results: Whole mouth and individual mucosal sites for *Candidal* counts increase significantly during treatment with upper removable orthodontic appliances.

Conclusion: The results suggest that removable orthodontic appliances treatment promotes an increase in *Candida* counts. Furthermore, removable appliance therapy had a positive transient influence upon the prevalence and density of oral candidal carriage. This can indicate a more cautious approach when providing orthodontic treatments for immunocompromised children regarding the increased possibility of candidal infection.

Keywords: Removable appliance, Colonization, *Candida*. (J Bagh Coll Dentistry 2016; 28(3):137-141).

INTRODUCTION

Malocclusions are known as the 3rd most common oral health problems, which caused a number of complications ⁽¹⁾. The advent of orthodontic treatment became increasingly popular for correcting these complications ⁽²⁾.

Patients undergoing orthodontic treatment wear either Removable Orthodontic Appliance (ROA) or Fixed Orthodontic Appliance (FOA), and at the beginning orthodontic procedures were considered non-invasive, but after that they found that these appliances can be associated by difficulty in cleaning during treatment, retentive areas created that favor biofilm accumulation and bacterial growth ⁽³⁾.

Further studies showed that wearing orthodontic appliances brought about several intraoral changes, such as increased biofilm accumulation, elevated microbial colonization, potential enamel demineralization, alterations in saliva buffer capacity, and even caused a harmful effect on periodontal tissues ^(4,5).

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, therefore oral candidosis considered is an accepted complication of upper removable appliance (URA) wear. The density with which the oral mucosa is colonized by *Candida* is significantly increased in wearers of both fixed and removable orthodontic appliances ^(6,7).

Candida is recovered more frequently from certain sites in the mouths of URA wearers namely the anterior and posterior palate, anterior tongue and left buccal mucosa ⁽⁶⁾.

In terms of site, the highest prevalence of *Candida* carriage during orthodontic therapy with URA was the palate (much of which is covered by the URA), despite the fitting surface of the appliance itself has been found to be more densely colonized by *Candida* than any of the mucosal sites sampled during the period of URA wear ⁽⁷⁾.

Hibino *et al.* ⁽⁸⁾ reported *C. Albicans* as an opportunistic pathogen, which commonly isolated from the mouth of orthodontic patients with removable appliances, formed *Candida* biofilm. These microorganisms in the biofilm sometimes may enter into blood stream and cause candidemia.

During orthodontic therapy with upper removable orthodontic appliances, significant increase in the prevalence of *Candida* carriage has been noted ⁽⁷⁾. Although, there are several investigations in medical literature studied the effect of fixed orthodontic appliances on oral *Candida* colonization ⁽⁹⁻¹¹⁾. More researches are needed for investigating oral *Candida* carriers in patients with removable orthodontic appliances ⁽¹²⁾, and it is also important to determine the oral microbial alteration in patients undergoing orthodontic treatment in order to maintain the oral health of the patients, therefore; the aim of this study was to investigate the effect of upper removable orthodontic appliances on the mucosal *Candida* count in group of apparently healthy orthodontic patients.

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MATERIALS AND METHODS

Twenty-four patients 13 males and 11 females aged 8-12 years old undergoing orthodontic treatment with Upper Removable Appliances (URAs) were selected from patients attending the Department of Orthodontics / College of Dentistry / Baghdad University.

The medical history of each individual was checked for factors known to affect carriage of oral Candida, i.e. diabetes, anemia and immunosuppression. Similarly, individuals who had received or were currently receiving treatment with antibiotics, antifungals or steroids in the previous three months were excluded from the study. None of those included had previously experienced orthodontic treatment or worn any type of oral prosthesis. URAs were worn for at least 4 weeks, continuously day and night.

Appliances were brushed with tooth brush and tooth paste as an oral hygiene measure. Appliances were in passive contact with mucosa, although slight pressure would result from chewing.

To standardize the patient population, all upper removable appliances were made by the same technician and they were made of an acrylic base, retention elements and active element (Adams' clasp, Hawley arch and active element Z-spring). The selected patients were instructed to brush and use dental floss three times a day. For standardization, the oral hygiene of all the patients was provided by their parents throughout the study.

The oral candidal carriage of the subjects were taken from six intraoral mucosal sites (anterior palate, posterior palate, anterior tongue, posterior tongue, left cheek, right cheek) using the imprint culture method⁽⁷⁾.

In brief, sterile foam pads soaked in Sabouraud's broth were applied to each mucosal surface and then placed with the contact side down on Sabouraud's agar (Oxoid). The agar plates were then incubated aerobically at 37°C for 48 hr., the foam pads were removed, and the plates were re-incubated for an additional 72 hr. The candidal colonies were counted separately for each site by visual examination and expressed as colony-forming units (CFU)/mm² (13).

Swabs from the patients were taken three times during the orthodontic treatment, the first sample was taken before the insertion of upper removable orthodontic appliance (T1); the second sample was taken after 14 days (T2); and lastly the third sample taken after 28 days (T3).

Statistical analyses

Data were analyzed using SPSS (Statistical Package of Social Science) software version 19.

In this study the following statistics were used Descriptive statistics; including medians, means, standard deviations, minimum and maximum values and statistical tables. Inferential statistics including:

- Shapiro-Wilk test: To test the normality of the distribution of the data.
- Wilcoxon signed ranks test: To detect side difference.
- Kruskal-Wallis H test: To compare the measured colony forming unit among the regions and durations
- Mann-Whitney U test: To test the gender difference and after Kruskal-Wallis H test to test any statistically significant difference between each two regions and durations.

In the statistical evaluation, the following levels of significance are used:

Non-significant	NS	$P > 0.05$
Significant	S	$0.05 \geq P > 0.01$
Highly significant	HS	$P \leq 0.01$

RESULTS

Firstly, data were checked for normality of distribution using Shapiro-Wilk test and the results indicated that the data were not normally distributed, so non – parametric tests were used.

Comparison the right and left sides and the anterior and posterior areas in different regions was performed using Wilcoxon signed ranks test and the results revealed non-significant difference in each gender so, the right and left and the anterior and posterior areas were pooled.

Using Mann-Whitney U test, gender difference was evaluated and the results indicated non-significant gender difference, hence the data of both genders were pooled also and effects of different areas and durations were assessed using Kruskal- Wallis H test then Mann-Whitney U test.

Descriptive statistics and region difference of colony forming unit in each period was presented table (1). There was highly significant regions' difference in T1 and T2 only.

Comparison of each two regions in these periods revealed highly significant difference between cheek and tongue and between palate and tongue (Table 2).

Descriptive statistics and duration difference of colony forming unit in each region was demonstrated in table (3). Generally, the median values increased dramatically from T1 to T3 with a highly significant difference.

Comparing each two periods revealed a highly significant difference in all regions.

Table 1: Descriptive statistics and region difference of colony forming unit in each period

Durations	Regions	Descriptive statistics						Region difference	
		N	Median	Mean	S.D.	Min.	Max.	X ²	p-value
T1	Cheek	48	1	0.56	0.58	0	2	28.273	0.000 (HS)
	Palate	48	0	0.52	0.77	0	3		
	Tongue	48	1	1.25	0.73	0	3		
T2	Cheek	48	2	1.77	1.24	0	4	17.780	0.000 (HS)
	Palate	48	2	1.75	1.21	0	4		
	Tongue	48	3	2.71	1.03	1	5		
T3	Cheek	48	3	3.54	1.56	0	6	2.296	0.317 (NS)
	Palate	48	3	3.15	1.47	1	6		
	Tongue	48	3	3.52	1.52	0	6		

Table 2: Comparing each two regions in each period

Durations	Cheek vs. Palate		Cheek vs. Tongue		Palate vs. Tongue	
	MW	p-value	MW	p-value	MW	p-value
T1	1042	0.362 (NS)	583	0.000 (HS)	567	0.000 (HS)
T2	1137	0.91 (NS)	684	0.000 (HS)	654	0.000 (HS)

MW= Mann-Whitney U test

Table 3: Descriptive statistics and duration difference of colony forming unit in each region

Regions	Durations	Descriptive statistics						Durations' difference	
		N	Median	Mean	S.D.	Min.	Max.	X ²	p-value
Cheek	T1	48	1	0.56	0.58	0	2	73.118	0.000 (HS)
	T2	48	2	1.77	1.24	0	4		
	T3	48	3	3.54	1.56	0	6		
Palate	T1	48	0	0.52	0.77	0	3	67.973	0.000 (HS)
	T2	48	2	1.75	1.21	0	4		
	T3	48	3	3.15	1.47	1	6		
Tongue	T1	48	1	1.25	0.73	0	3	62.596	0.000 (HS)
	T2	48	3	2.71	1.03	1	5		
	T3	48	3	3.52	1.52	0	6		

Table 4: Comparing each two periods in each region

Regions	T1-T2		T1-T3		T2-T3	
	MW	p-value	MW	p-value	MW	p-value
Cheek	513	0.000 (HS)	109	0.000 (HS)	462.5	0.000 (HS)
Palate	479	0.000 (HS)	133.5	0.000 (HS)	568	0.000 (HS)
Tongue	321	0.000 (HS)	229.5	0.000 (HS)	767.5	0.003 (HS)

MW= Mann-Whitney U test

DISCUSSION

The oral cavity presents countless species of microorganisms particularly Candida, which are frequently seen in the oral cavity of almost half of the healthy population, and may be associated with orthodontic appliance contamination and pathologies. Wearing orthodontic appliance, is also known as a risk factor, can promote colonization of Candida and result in oral candidiasis⁽¹⁴⁾.

In this study comparison in each duration among cheek, palate and tongue revealed a high significant difference in T1 and T2. This result is comparable to that reported by Arendorf and Addy⁽⁷⁾ who found an increase in candidal counts

at 5 months of removable orthodontic appliance use, and considering that the counts further increased in regions where an appliance was being worn. They also noted that the candidal counts increased in regions where no appliance was present.

The results of this study showed that the difference is non-significant for T3 duration while it is highly significant for T1 and T2 duration this may be attributed to relationship between Candida and the resistance of patients, where an increase in the Candida population may cause a temporary weakening of the resistance of the body during the initial phase after the application of the ROA. The amount of candida

gradually declined, which may be due to the gradual adaptation of the patients to the new oral environment. Regional differences may also contribute to the variations observed. Moreover, full recoveries of systemic and local resistance were also observed. The increase in the number of *Candida* colonies may be associated with an increase in the amount of plaque and microbes in the oral cavity of patients shortly after ROAs start to be worn.

In this study comparison in each region (cheek, palate and tongue) among different durations, revealed a high significant difference with the highest median values for all the three regions were found during T3 where the lowest were found at T1, this agreed with Arendorf and Walker⁽¹³⁾ who found that the presence of a prosthesis or an appliance increase candidal numbers, not only at the occluded site, but at all mucosal sites sampled.

Arendorf and Addy⁽⁷⁾ investigated 33 patients who underwent ROA therapy and found a direct relationship between the presence of ROA and candidal species.

The incidence of oral *Candida* among normals prior to the application of removable appliances was lower than that reported after 28 days. This may be attributed to the fact that the patients brushed their teeth accurately prior to sampling, thus reducing the number of microbes in the oral cavity. The rate of pathogenesis and number of colonies of oral *Candida* significantly increased compared with those prior to treatment. These findings may be due to the ROAs resulting in a lowering of the local defense mechanism of oral mucosal cells. Oral mucosal cells, which act as mechanical barriers, and metabolism play important roles in increasing the resistance of the mouth to infection. Thus *Candida* can easily adhere to any damage in the oral epithelia⁽¹⁵⁾.

Adhesion of *Candida* to the parts of the ROA, may affect colony formation. The extent of adhesion is dependent on the surface roughness and type of material used. The adhesion of *Candida* to the surface of orthodontic appliances should be studied further.

There is also a relationship between *Candida* and the resistance of patients, where an increase in the *Candida* population may cause a temporary weakening of the resistance of the body during the initial phase of application of the ROA.

There are few published studies to be compared with the present study. Addy *et al.*⁽⁶⁾ using Imprint cultures to the mucosa of URA wearers and non-wearers recorded prevalence of 52 and 46% respectively.

A later study, utilizing the imprint culture technique to sample of mucosal surfaces and URAs demonstrated an increase in the prevalence of *Candida* from 39.4 to 78.8% when URAs were worn⁽⁷⁾. It is believed that URAs increase the risk of oral carriage of yeasts by providing an ecological niche^(6,7).

C. Albicans and other *Candida* species readily adhere to oral epithelia that are covered by acrylic dental plates and to the surface of the acrylic plates in contact with the mucosa^(7,15). Long-term wearing of dental removable appliances is a major factor for the colonization of *Candida* on mucous surfaces; this colonization may lead to levels sufficiently high to give rise to oral candidosis particularly affecting the mucosa beneath the appliance aid⁽⁶⁾. Other studies have shown that oral candidosis is associated with a high density of yeast determined by imprint culture⁽⁵⁾.

Multiple areas of the oral mucosa including the dorsum of tongue, the main reservoir for *Candida* in the oral cavity, been sampled by imprint culture the sensitivity of this isolation method would have been significantly higher⁽⁵⁾.

The site prevalence and intra-oral density of candidal organisms may be increased by local factors including prosthesis. However, the prevalence of candidal recovery at some sites and candidal densities at all sites were significantly increased in both fixed and removable appliance wearers and that there seems to be a direct relationship between the presence of a removable appliance, *Candida* and low salivary pH levels. It is important to emphasize that no healthy patients developed *Candida* infection from the orthodontic appliances. However, there seems to be at end that some non-*Candida* carriers converted to *Candida* carriers following the insertion of the appliances by unknown mechanism. This may indicate a more cautious approach when providing orthodontic treatments to immunocompromised children concerning the possible increased risk of candidal infection.

Orthodontic appliances may favor the adherence of *Candida* to epithelial cells but do not influence the presence of them in saliva. The findings of previous studies therefore suggest that the increase in candidal counts is attributable to poor oral hygiene.

This hypothesis is supported by the findings of this study of multiple positive significant correlations between the candidal counts and removable appliance wearers. Furthermore, the positive correlation between the candidal counts observed in the removable appliance group at T3 may also be another indication of a possible

relationship between poor oral hygiene and candidal presence.

In conclusion, treatment of malocclusions using removable appliances may prepare new stagnant areas susceptible for colonization and retention of *Candida* species.

Patients should be motivated each visit for oral hygiene during their orthodontic therapy.

REFERENCES

1. Castellanos-Cosano L, Machuca-Portillo G, Mendoza-Mendoza A, Iglesias-Linares A, Soto-Pineda L, Solano-Reina E. Integrated periodontal, orthodontic, and prosthodontic treatment in a case of severe generalized aggressive periodontitis. *Quintessence Int* 2013; 44: 481-5.
2. Ratson T. Diagnosis and treatment of supernumerary teeth in the premaxillary region: a literature review. *RefuatHapehVehashinayim* 2013; 30: 26-30
3. Lee SJ, Kho HS, Lee SW, Jang WS. Experimental salivary pellicles on the surface of orthodontic materials. *Am J Orthod Dentofac Orthop* 2001; 119: 59-66.
4. Condo R, Casaglia A, Condo SG, Cerroni L. Plaque retention on elastomeric ligatures. An in vivo study. *J Oral Implantol* 2013; 5: 92-9.
5. Darodeh N, Shehabi A, Al-Bitar Z, Al-Omari L, Badran S, Al-Omiri M, et al. *Candida* colonization in patients with fixed orthodontic appliances: The importance of some nutritional and salivary factors. *Afric J Microbio Res* 2011; 5: 2150-54.
6. Addy M, Shaw WC, Hansford P, Hopkins M. The effect of orthodontic appliances on the distribution of *Candida* and plaque in adolescents. *Br J Orthod* 1982; 9: 158-63
7. Arendorf TM, Addy M. Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. *J Clin Periodontol* 1985; 12: 360-8.
8. Hibino K, Wong RW, Hagg U, Samaranayake LP. The effects of orthodontic appliances on *Candida* in the human mouth. *Int J Paediatr Dent* 2009; 19: 301-8.
9. Arslan SG, Akpolat N, Kama JD, Ozer T, Hamamci O. One-year follow-up of the effect of fixed orthodontic treatment on colonization by oral *Candida*. *J Oral Pathol Med* 2008; 37: 26-9.
10. Saloom HF, Mohammed-Salih HS, Rasheed SF. The influence of different types of fixed orthodontic appliance on the growth and adherence of microorganisms (in vitro study). *J Clin Exp Dent* 2013; 5: 36-41.
11. Sukontapatipark W, el-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances: A scanning electron microscopy study. *Eur J Orthod* 2001; 23: 475-84.
12. Hibino K, Wong RW, Hagg U, Samaranayake LP. The effects of orthodontic appliances on *Candida* in the human mouth. *Int J Paediatr Dent* 2009; 19: 301-8
13. Arendorf TM, Walker DM. Oral candidal populations in health and disease. *Br Dent J* 1979; 147: 267-72.
14. Barbeau J, Seguin J, Goulet JP, de Koninck L, Avon SL, Lalonde B, et al. Reassessing the presence of *Candida albicans* in denture-related stomatitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003; 95: 51-9.
15. Odds FC. *Candida* and candidosis, a review and bibliography. London: Bailliere Tindall; 1988.