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Original Research Article

Effect of Removeable Orthodontic Appliance on Oral PH, Candida Albicans, Candida Dubliniensis, and Streptococcus Mutans Count

Farrokh Kolahi Aval[1], Negar Moghaddasi[2], Tara Azimi[3], Zahra Nematollahi[4], Abdolreza Jamilian[5*]

[1]DDS, Laguna Niguel, CA, USA
[2]DDS, Los Angeles, CA, USA
[3]DDS, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]D.D.S, Dental Research Center, Research Institute of Dental Science, Shahid Beheshti University of Medical Science, Tehran, Iran, and CranioMaxillofacial Research Center, Azad University, Dental Branch, Tehran, Iran
[5]Department of Orthodontics, Faculty of Dentistry, Cranio Maxillofacial Research Center, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

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Abstract: Removable orthodontic appliances (ROAs) are popular devices to move or retain teeth during or after orthodontic treatments providing a good environment for adhesion and colonization of pathogenic and non-pathogenic organisms. The aim of the present study was to explore the presence and variability of oral PH, levels of Candida Albicans, Candida Dubliniensis, and Streptococcus (S.Mutans) in children before and during the treatment with ROAs. In this clinical trial study, a total of 160 patients aged 8–12 years old in both genders were enrolled from a larger sample of patients who were clinically confirmed to obtain ROAs. They were randomly divided into three groups; 1- PH Group (n=51), 2- Candida Group (n=51), and 3- S.Mutans Group (n=58). Patients were assessed prior (T_0) and again one month later (T_1) following appliance insertion. The oral cavity was sampled for PH level, Candida species and bacterial species by culture. Paired t-Test and ANOVA were applied for statistical analysis. The level of significance was assumed to be $P \leq 0.05$ for all tests. In Group 1, PH values decreased from 6.89±0.5 in T_0 to 6.55±0.7 in T_1 (0.5% decrease) with statistically significant difference (P=0.002). In group 2, total Candida count at dorsal tongue was more than hard palate. Also, the difference (5.1±6 increase) was significant between the mean Candida count in T_0 and T_1 (P=0.001). Although the C.Albicans count was more than C.Dubliniensis, the difference was not significant. In group 3, S.mutans showed a significant difference between the two subgroups of case and control (P<0.005). ROAs change the balance to decrease the values as well as increase the proliferation/colonization of Candida specimen and S.mutans. This implicated the importance of paying special attention to oral hygiene in orthodontic patients to prevent oral disease and the aggravation of systemic disease in immunocompromised conditions.

Keywords: Candida Albicans; Candida Dubliniensis; Caries; Oral; Orthodontic Appliances, Removable; Streptococcus Mutans.

INTRODUCTION

Thanks to the development of various orthodontic appliances and protocols, an increase in prevalence of orthodontic treatments has been reported to meet the particular needs of each patient [1-3]. Removable orthodontic appliances (ROA) are popular devices to move or retain teeth during or after orthodontic treatments, providing a good niche for adhesion and colonization of pathogenic and non-pathogenic organisms and facilitating infection [4, 5].

Different types of Candida specimens are considered as opportunistic agents locating in the oral cavity and classified into eight categoreies: Candida albicans (C.Albicans), Candida tropicalis, Candida glabrata, Candida parapsilosis, Candida krusei, Candida kefyr, Candida stellatoidea, and Candida dubliniensis (C.Dubliniensis) [6, 7]. Common types among them are C.Albicans, and C.Dubliniensis which could proliferate in patients with suppressed immune system and become pathogenic in spite of their limited virulence [5, 8]. The

***Corresponding Author:** Abdolreza Jamilian, Email: info@jamilian.net
Department of Orthodontics, Faculty of Dentistry, Cranio Maxillofacial Research Center, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

proportion C.Dubliniensis/C. Albicans is reported to be 1 to 5% [9].

Previous literature reviews reported moderate-to-high evidence of significant effect of orthodontic appliances on the concentration of oral microorganisms, resulting in changes of S.Mutans and Lactobacilli [10]. S.Mutans is reported to be the most cariogenic bacteria due to its acid production and tooth adhesive characteristics [11].

The purpose of this clinical trial study was to evaluate the changes in oral PH, the levels of C.Albicans, C.Dubliniensis, and S.Mutans in patients undergoing treatment with ROA. The data may stress the importance of a careful monitoring of patients treated orthodontically for risk of caries development.

MATERIAL AND METHODS

The present clinical trial study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Azad University, Tehran, Iran. The study population consisted in 160 patients ranging from 8 to 12 years old.

Including Criteria

All patients referred to the orthodontic department to treat with ROAs, living in the same geographic area, without preventive treatment with fluoride, and no history of hospitalization during the last six months.

Excluding Criteria

History of systemic or metabolic diseases, growth disorders, cognitive handicaps, congenital diseases, oral infection, use of antibiotics, or antiseptic/ antibacterial mouth rinse, decreased saliva flow (dry mouth syndrome).

Informed consent was obtained from all subjects. Then, the participants randomly divided into three groups;

Group 1: Oral PH (58 subjects according to similar articles)

The pH values were measured with portable pH meter strips. Before wearing the orthodontic plaque, the PH strip was placed on the floor of patient's mouth, then removed after one minute. The appeared colors were matched on the color scale and values was recorded.

Group 2: Candida (Albicans and Dublininsis); (58 subjects)

The Candida count was evaluated using the standardized technique of "imprint culture" introduced by Arendorf and Walker in 1979. In brief, sterile foam pads (1cm ×1cm) were dipped in Sabouraud's broth, then placed on the mucosal tissue of posterior hard plate and dorsal tongue. The pads were pressed on to a Sabouraud's agar plate (Hampshire, UK), then incubated at 37º for 8 hours (h). The plates were incubated at 37º for 72 h followed by the removal of pads from the plates. Candida numbers were determined by direct visual counting, and expressed by colony forming units per mm^2 (CFU/mm^2).

Yeast specimen (Albicans and Dublininsis) was determined using "germ tube test". Briefly, randomized colonies were incubated at 37 ºC for 2 h in pooled human serum, and then sub cultures from colonies on new plates incubated at 45ºC for 48 h. Under this circumstances, C.Albicans produces short, slender, and tube-like structures (germ tubes). On the other side, yeast isolated that were unable to growth at this temperature were determined as C.Dublininsis.

Group 3: S.Mutans (58 subjects);

The study population of this group consisted in 58 participants ranging from 8 to 12 years old whom randomly divided into two groups of case and control. Both groups were evaluated for the number of decayed teeth in clinical examination, and then the values were recorded in a table. For S.Mutans colonies identification, the area was isolated using cotton rolls. The sample was collected from the hard palate beside the gingival margin of molar teeth using a sterile swap. The samples were immediately placed in 4 mL of Stewart's transport medium (Serial No: 105418, Merck Co., Germany). For identification and counting of S. mutans colonies, the samples were transferred to Mitis Salivarius agar (Serial No: 1686540, Biomark co., India), then incubated for 48 h at 37°C under anaerobic conditions following culture in the CO_2 pack.

The suspected colonies were biochemically tested (microscopic assessment by Gram staining: purple stains, and then catalase test: negative results were accepted as S.Mutans colonies). Finally, number of the colony forming units (CFUs) per milliliter was calculated using this formula:
CFUs=number of counted colonies × inverse of the dilution

Colonies were counted using a colony counter. The dilution factor is inverse of the dilution of culture medium for bacterial counting.

Statistical Analysis

Paired t-Test and ANOVA were applied for statistical analysis. The level of significance was assumed to be $P \leq 0.05$ for all tests.

RESULTS

Group 1:

Among 58 participants in Group 1 at T_0, seven individuals excluded from the study due to medication consumption (3), appliance fracture (1), no refer (3), losing the appliance (1). Finally, the study was continued with 51 persons with the mean age of

10.4±1.9 years old; 34 (66.8%) female and 17 (33.3%) male.

PH values decreased from 6.89±0.5 at T_0 to 6.55±0.7 at T_1 (one month later). There was a 0.5% decrease in PH index following appliance placement. The difference was statistically significant (P<0.002).

Group 2:

Out of 58 participants at T_0, seven of them were excluded because of medication consumption (4) and appliance fracture/lost (3). Finally, a total of 51 individuals with the mean age of 10.4±1.9 years old, 34 (66.8%) female and 17 (33.3%) male were enrolled at T_1 (one month later). Values of Candida counts at different areas of oral environment are reported as mean ± standard deviation in Table 1.

Table 1: The effect of removeable orthodontic appliances on total Candida count of oral environment

	Mean ±SD at T_0	**Mean±SD at T_1**	**Δ: T_1-T_0**	**Δ (%)**	**P-value**
Hard Palate	6.8±12.6	14.9±11.4	+8±8.4	120	0.001*
Dorsal Tongue	8.1±12.1	16.4±13.7	+8.3±7.5	102	0.001*
Total Candida Count	14.9±23.2	37.6±31.2	+22.7±16.4	152	0.001*
Mean Candida Count	7.5±11.6	12.6±10.4	+5.1±6	68	0.001*
P<0.05, Paired t-test, as appropriate.					

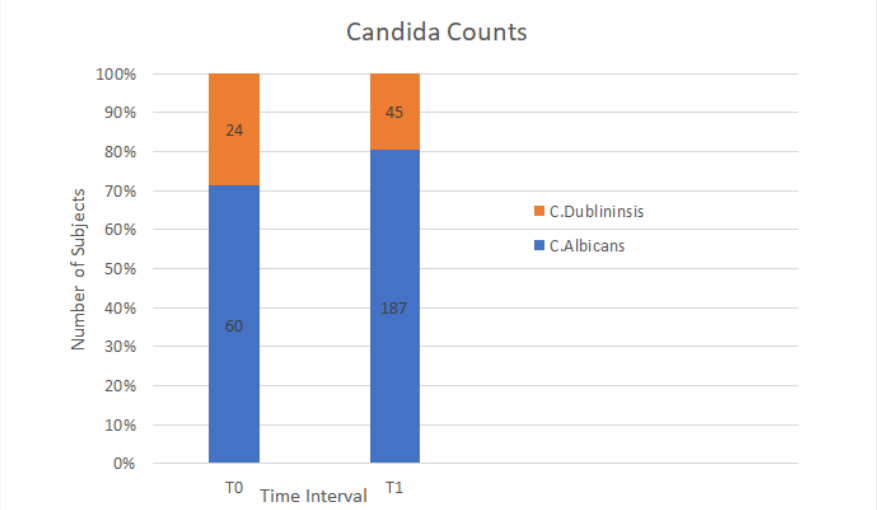

Fig 1: Candida Specimens count at baseline (T_0) and one month later after ROA utilization (T_1)

Group 3:

The study population of this group consisted in 58 participants ranging from 8 to 12 years old whom randomly divided into two groups of case and control.

The case group included 28 orthodontic children (14 males, 14 females; mean ag of 10.2±1.2) who had been carrying removable appliances. The control group comprised 30 children (15 males and 15 females; mean age of 9.7±3.8) who had never undergone orthodontic therapies. Results are detailed in Table 2. Data analysis using ANOVA test showed a significant difference between the two groups of case and control (P=0.000).

Table 2: Characteristics of S.Mutans groups

	Mean±SD of age	**Mean±SD of Decayed Teeth**	**Mean±SD of S. Mutans Colonies**
Case (n=28)	10.2±1.2	0.2 ±5.2	1181.36±720.89
Control (n=30)	9.7±3.8	2.2±5.4	491.13±421.92
Total (n=58)	9.95±0.25	1.2±1	824.34±676.60

DISCUSSION

Orthodontic devices act as a trap and play an important role in gingival-periodontal diseases and dental caries, since they facilitate the accumulation of yeasts and other microorganisms to their surfaces, whether acrylic, glass, composite, sealants, or membranes, in terms of quantity and type/diversity, altering the oral microbiota [12]. Several functional/orthodontic devices are used in interceptive orthodontics, including those patients needing rapid maxillary expansion (RPE). We evaluated the microbial changes associated to these types of appliances.

PH

We observed a significant decrease in PH index which was similar to the previous studies including Arab *et al*., [13] that implicated a significant

decrease in PH index during fixed orthodontic treatment.

Candida

We observed a significant increase in the level of C.Albicans, however, the amount of C.Dublininsis didn't show a statistically significant increase following ROAs. This result is in line with several studies reporting a high and significant prevalence of C.Albicans yeast in orthodontic patients.

In a literature review by Hnino *et al*., [14], they implicated that the most common Candida species reported in the orthodontic patients was C.Albicans. However, some evaluations have refuted the hypothesizes of a significant high prevalence of Candida specimens in patients undergoing orthodontic treatment [15]. This might be due to the number of subjects and the method of fungal detection. Assessing the Candida count base on culture may lead to underestimate those yeasts that are not easy to cultivate, especially if present in low percentages. Further studies using high throughput technologies instead of culture-based methods are desirable to analyze the real specimens and counts of oral mycobiome among orthodontic patients.

Although no development of Candida infection occurred in healthy individuals, several non-Candida carriers converted to Candida carriers after utilization of orthodontic appliances through unknown mechanism. This indicates cautions of orthodontic treatment in immunocompromised patients concerning the possible risk of candida infection [16].

Muggiano *et al*., [16] implicated orthodontic appliances as favor places for Candida specimen to adhere epithelial cells without effect on Candida presence in saliva. Also, the amount of anti-Candida albicans IgA didn't show correlation with yeast adherence or Candida presence in the oral environment.

Overall, we observed a significant low PH level of oral environment, and a high significant increase in C.Albicanse and S.Mutans count after ROAs among our patients.

Previous studies declared a probable direct linear relationship between the presence of ROAs, C.Albicans and salivary PH levels [14, 16].

Regarding more prevalence of opportunistic bacteria and fungi in orthodontic patients in comparison with non-orthodontic patients, special attention to oral hygiene in patients with ROAs is emphasized to prevent further periodontal problems and the aggravation of systemic disease in immunocompromised conditions [16].

Previous assessments showed that the acrylic ROA predispose proliferation of Candida species regardless the status of the host immune system due to provide a growth environment for C. albicans through an extended coverage of oral mucosal by wearing several hours in a day for a long time [4, 14, 17, 18]. These occurred due to two important factors; first; the inhibition of the C.Albicans removal from mechanical irrigation of saliva in presence of ROA, and secondly, binding the yeast to the acrylic surface of the ROA through the hydrophobic effect and van der Waals forces. All of aforementioned reasons change the balance to increase the proliferation and colonization of Candida specimen [19, 20].

S.Mutans

Ortu *et al*., (21) reported a significant increase in the level of S.Mutans and Lactobacillus during important phases of the rapid palatal expansion (with RPE or Mc Namara expander) in children aged 6–9 year. Also, Kundu *et al*., [4] reported statistically significant levels of S.Mutans, Lactobacillus sp., and C.Albicans in both groups of removeable or fixed orthodontic appliances at all intervals of 1-,3-, and 6-months among children aged 6-15 years.

Using confocal laser scanning microscopy and transmission electron microscopy, Sampaio et al. [11] reported the interaction between the S.Mutans cells and C. Albicans throughout polysaccharides of the biofilm matrix. Bachtiar EW and Bachtiar BM [22] implicated the role of Candida species in the onset of dental caries due to their synergistic relationship with S.Mutans which are commonly associated in mature plaque, particularly in children. C. albicans is reported to increase the cariogenic potential of the S.Mutans biofilm, and consequently dentine demineralization [11].

We evaluated the changes of Candida and S.mutans in oral cavity one month after the utilization of ROAs and observed significant increase of them among our patients. Contaldo *et al*., [15] implicated that the qualitative and quantitative changes occurred as early as seven days which became more consistent three months following the orthodontic appliances wearing, with stable colonization first by orange and then by red species. However, ROAs were less associated with worsening of periodontal indices and caries because they can be easily removed to allow for proper oral hygiene [15]. Despite, Lucchese *et al*., [23] found that the major changes occurred through the first 15 days of treatment, independently from the type of orthodontic appliance.

Therefore, periodic clinical follow ups during the first month of treatment process affect the progression and maturation of periodontopathogen and cariogenic species in oral cavity.

CONCLUSION

Long-term utilization of orthodontic appliances may negatively affect the microbial flora including Candida specimens and S.Mutans, consequently, result in the higher risk of new carious lesions or periodontal problems among orthodontic patients. Follow-up sessions within short time intervals to motivate them for oral hygiene during their orthodontic therapy is highly recommended.

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Conflicts of Interest: None

Author Contributions:
Farrokh Kolahi aval: Investigation, Writing, Formal Analysis
Negar Moghaddasi: Data Collection
Tara Azimi: Data Collection
Zahra Nematollahi: Writing, Original Draft Preparation
Abdolreza Jamilian: Conceptualization, Supervision

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