

## Comparison of salivary *Candida* profile in patients with fixed and removable orthodontic appliances therapy

Elham Khanpayeh<sup>1</sup>, Abbas Ali Jafari<sup>2</sup>, Zohre Tabatabaei<sup>3</sup>

<sup>1</sup>School of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

<sup>2</sup>International Branch, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

<sup>3</sup>Orthodontist, Private Practitioner, Safaieh, Yazd, Iran.

Received: June 2013, Accepted: April 2014

### ABSTRACT

**Background and Objectives:** Long-term usage of fixed and removable orthodontic appliances creates a favorable environment for the augmentation of oral normal microflora particularly *Candida* species, which can increase the risk of periodontal lesions. The aim of this study was to assess quantitative and qualitative alterations in the carrier rate of *Candida* spp. after placement of fixed and removable orthodontic appliances on permanent dentition.

**Materials and Methods:** Patients enrolled in this study were children aged 7-18 years, who having fixed or removable orthodontic appliances, attended in orthodontics clinic for periodical provision. Six months after beginning of their orthodontic therapy, saliva samples were collected and cultured on Sabouraud dextrose agar for identification and enumerating of isolated *Candida* colonies. *Candida* species were identified using the germ tube test and API 20C AUX identification system. Data was analyzed with T-test and Chi square using SPSS 17 software.

**Results:** The average number of *Candida* colonies isolated from saliva in patients with fixed orthodontic was more than patients with removable appliance ( $P=0.001$ ). Also frequency of non-albicans *Candida* species was higher in patients with fixed orthodontic appliances in compare with fixed group ( $p=0.001$ ).

**Conclusions:** The results suggest that fixed orthodontic appliances treatment promotes an increase in salivary *Candida* carriers particularly non-albicans *Candida* species in compare with removable ones. This can indicate a more cautious approach when providing fixed orthodontic treatments for immunocompromised children regarding the increased possibility of candidal infection.

**Keywords:** Orthodontic brackets, Colonization, Saliva, *Candida Sp*

### INTRODUCTION

Since 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). Current orthodontic appliances include: fixed orthodontic

appliances and removable orthodontic appliances. However at the beginning orthodontic procedures were considered noninvasive, but 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 (3, 4).

Lucas *et al*, also isolated aerobic and anaerobic bacteria from blood samples of patients using orthodontic appliances as in the case of placement of a separator (5).

Intraoral prostheses such as orthodontic appliances

\*Corresponding Author: Dr. Abbas Ali Jafari, International Branch, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

E-mail: Jaabno@gmail.com

are also associated with some potential harm to teeth and periodontal tissues. For example maintaining of oral hygiene can be difficult during orthodontic treatment, which may lead to plaque formation and gingival inflammation (3).

*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 (6, 7) with average, 300 to 500 colony forming units per milliliter of saliva (8). 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 (9).

However, there are several investigations in medical literature studied the effect of fixed orthodontic appliances on oral *Candida* colonization (10-12) with controversy results, more recently a literature review study confirmed that more researches are needed for investigating oral *Candida* carriers in patients with orthodontic appliances (13).

Hibino *et al.* 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 (13).

In another study Thornberg *et al.* reported changes in 8 microbial periodontal pathogen levels before, during, and after orthodontic treatment. Their study showed that significant increasing of high pathogen level after 6 months insertion the fixed orthodontic appliances, and return to normal pretreatment levels after than 12 months. According to their results, fixed orthodontic treatment induced alteration in the levels of periodontal pathogens during and after treatment period, but this effect is transitory and the periopathogen levels, will decrease to normal value after one year of treatment (14).

It is important to determine the oral microbial alteration in patients undergoing orthodontic treatment because in some cases they involved long treatment duration, which the clinicians are committed to maintain their oral health.

In Iran, using orthodontic treatment is more popular as Borzabadi *et al.* (2009) in an epidemiological study reported that about 36.1% of Iranian schoolchildren were in need for orthodontic treatment as a result of four prevalent severe occlusal traits including

increased overbite, increased overjet, severe maxillary and mandibular crowding (15). Therefore the aim of this study was to investigate the effect of fixed and removable orthodontic appliances on the salivary *Candida* colonization in the susceptible group of apparently healthy orthodontic patients.

## MATERIALS AND METHODS

Totally 80 male and female subjects aged 7-18 years, including 40 using orthodontic fix appliance (full band and bond) with pre-adjusted metal brackets and 40 patients having removable orthodontic devices, six months after beginning of orthodontic treatment, were entered in present study. All fixed appliances were banded by one orthodontist and all removable appliances were also made by the same laboratory. The selected patients were all instructed to brush and use dental floss 3 times a day, after beginning of orthodontic therapy. Subjects, who reported history of any oral and systemic diseases or intakes of medications, were excluded from this study. The nature of the research was explained to the patients and their parents as well as all participants were firstly signed a consent form and then prepared for salivary sample collection.

**Salivary sample collection.** Subjects were instructed not to eat or to drink for at least 2 hours before sample collection and also to brush their teeth once in the morning on the day of salivary collection. Un-stimulated whole saliva collection was carried-out using sterilized plastic receptacles with lids. Patients were instructed to expel saliva into the receptacle until a volume of approximately 3 ml was collected. Saliva was transported in a thermal receptacle with ice, and was processed within 2 hours after collection. 100 µl of saliva then aseptically seeded on Sabouraud dextrose agar (Merck, Germany) plates supplemented with chloramphenicol (0.05g/l). The plates were incubated at 37°C and inspected after 24 and 48 hours.

**Identification of *Candida* species.** The number of isolated *Candida* colonies on the culture plate were manually counted for each plate, multiply by the dilution factor and expressed in number of colony forming unit per milliliter of saliva (CFU/ml).

*Candida* species were identified by performing the germ tube test, hyphae/pseudohyphae and

chlamydoconidia growth on Corn meal agar (16, 17) as well as using the identification system API 20C test (18).

**Germ tube test.** The germ tube test was performed based on standard method by incubation of a single *Candida* colony in pooled human serum at 37°C for 3 hours (16). A standard strain of *Candida albicans* was also used as positive control. Observing germinated yeast cells under microscope showed *Candida albicans* in this method.

**Corn meal agar.** Simultaneously the isolated *Candida* colonies were cultured on to Corn meal agar (Oxoid, Uk) for chlamydoconidia, blastoconidia, pseudomycellium formation and pattern of chlamydoconidia & blastoconidia formation (17). Slide preparation was then prepared and *Candida* species were determined as followed:

Large, thick-walled chlamydoconidia, usually terminal and present singly or in small clusters along with clusters of round blastoconidia represents as *Candida albicans*.

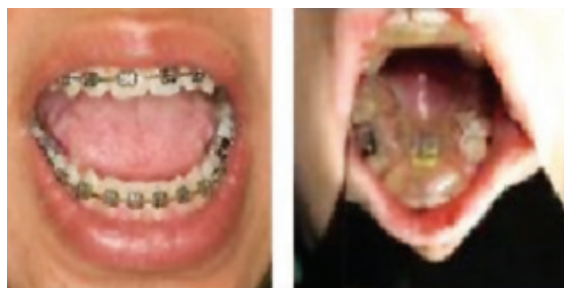
Oval blastoconidia singly or in small groups all along, long pseudohyphae represents as *C tropicalis*.

Short, pencil-like pseudohyphae with blastoconidia arranged singly along pseudohyphae known as *C parapsilosis*. Only yeast cells (blastoconidia) known as *C. krusei*.

Pseudohyphae with blastoconidia forming cross-match stick appearance represent as *C.glabrata*.

**API 20C method.** API tests were performed according to the manufacturer's instructions (bioMérieux Vitek, France). Ampoules containing the API20 C basal medium were melted at 50°C and used for *Candida* inoculation suspension preparation. *Candida* suspension was inoculated into each of the 20 plastic strips on the API strip using sampler with disposable tips for evaluating of carbohydrates assimilation. The strips were placed in plastic trays containing 5.0 ml of water and were incubated at 30°C for 72 h. Wells showing turbidity significantly heavier than that of the negative control were considered positive for carbohydrate assimilation (18). Identification was made by generating a microcode and using the API 20C Analytical Profile Index.

**Statistical analysis.** Descriptive statistics and the  $\chi^2$  test were used to determine age and gender differences, respectively using SPSS software. Two



**Fig. 1.** Fixed (left) and removable (right) orthodontic appliances used in present study

sample T-tests were used when the examined values were parametric. Mann-Whitney and Kruskal Wallis tests were used when the examined values were non-parametric (gender and *Candida* species). Chi-square test was used when data were cross-tabulated. Differences between the groups was indicated by the Pvalue when was considered significant  $\leq 0.05$ .

## RESULTS

Salivary samples of 80 subjects including 45 female and 35 male were aseptically cultured on Sabouraud dextrose agar plates. There was not any statistical differences between sex ( $P= 0.652$ ) and age groups ( $P= 0.312$ ) for patients with fixed and removable orthodontic appliances (Table 1).

There was seen a higher colonization of *Candida* on salivary samples of patients with fixed orthodontic appliances in comparison with patients with removable appliance ( $P= 0.0001$ ). A higher colonization of non-albicans *Candida* species were also seen in saliva samples of subjects using fixed orthodontic appliances in compare with subjects with removable device ( $P= 0.001$ ). The negative saliva culture in patients with removable appliances was 22.5% whereas this was only 5% in patients with fixed appliances (Table 2).

A salivary career of *Candida* species for both groups was decreased by increasing the duration of insertion the orthodontic appliances (Fig. 1).

## DISCUSSION

It is known which *Candida* species in human disease is usually endogenous, and clinical infection can arise in subjects, who are carriers and become predisposed by illness, debility or immunocompromised (19). It is also well documented that the presence of fixed orthodontic appliances in patients' oral cavity

**Table 1.** Characteristic of subjects and results of their saliva culture

Orthodontic braces type	Fixed	removable	
Variables	Mean (SD)	Mean (SD)	P value
Age	12.3 (1.4)	11.7(1.6)	0.312
Duration of appliance			
Use Month	10.3 (3.7)	8.7 (2.1)	0.017
Colony counts	695.5 (213.2)	112.1 (54.3)	0.0001
Gender			
Female	24	21	0.326
Male	16	19	
Isolated C. species			
C. albicans	18	25	
Non-albicans Spp.	22	15	0.001

influences plaque accumulation resulted by the colonization of important periodontopathic bacteria and fungi and promote changes in their oral microbiota. Patients, who accept fixed appliance orthodontic therapy, need to understand and be aware of the consequences of their treatment and their oral health. On the other hand, accepting fixed buccal orthodontic appliances needs more cautious approach for patients' home care (20).

In the current study we compare salivary *Candida* colonization of patients using fixed and removable orthodontic appliances on their permanent dentition. A higher density of *Candida* colonization was detected in saliva samples of patients with fixed than patients, who used removable orthodontic appliances. Our study provided convincing evidence that the placement of a fixed orthodontic brace did affect the oral mycological flora and change the non-carrier subject into a carrier of *Candida* species. This observation is consistent with data from other reports (2, 13, 21). The main cause of increase in inflammation and dental plaque is presence of new retentive spaces (22), which may compromises oral

health by increasing plaque formation that is associated with a worsening of clinical parameters (23).

Hagg *et al.* also showed a higher colonization and plaques index of *Candida* in patients with fixed orthodontic appliances similar to present study (24). Balenseifen also reported a higher colonization of *Candida* and few bacteria in the oral cavity of patients with fixed appliances in compare with removable for all isolated microorganism including non-albicans *Candida* species the same as present study (25).

In contrast to the present results, Addy *et al.* (26) reported no significant effect of fixed orthodontic appliance therapy on the prevalence of oral candidal carriage. They used imprint culture technique for sampling oral *Candida* in their study whereas the whole saliva sample collection was employed in current study. In the former method, a number of areas may have to be sampled to represent the oral cavity. The whole saliva sample collection and colony forming unit (CFU) technique, which used in present study is a relevant and sensitive technique that usually used for estimating the oral *Candida* carriage and also for clinical diagnosis of oral

**Table 2.** Frequency of *Candida* species isolated from culture of 100 µl saliva samples of both groups.

Orthodontic braces type	Fixed	Removable
Culture results	N (%)	N (%)
No growth	2 (5)	9 (22.5)
<i>C. albicans</i>	18 (45)	25 (62.5)
<i>C. tropicalis</i>	8 (20)	3 (7.5)
<i>C. parapsilosis</i>	6 (15)	2 (5)
<i>C. Krusei</i>	4(10)	1(2.5)
<i>C. Kefyr</i>	2(5)	0 (0)
Total	40 (100)	40 (100)

candidiasis (27-30).

Dar-Odeh *et al.* (4) also in a study showed that metallic fixed orthodontic appliances did not encourage oral *Candida* colonization during the first four months study period, which is disagreeing with our results. However, they also used the whole saliva sample as current study, but their sample collection was conducted in one and 4 months after insertion of orthodontic appliances, whereas all samples were collected after six months in present study. However there was seen a higher salivary *Candida* carriers in patients with fixed orthodontic treatment in compare with removable, but *Candida* colonization rates was decreased with increasing the duration of insertion in both groups. Thornberg *et al.* also reported an increasing of oral *Candida* level after 6 months of insertion the fixed orthodontic appliances and decreased after than 12 months of treatment (14).

Orthodontic treatment, especially fixed appliances, caused specific alterations in the oral environment, including reduction of pH, elevation in dental plaques accumulation and increasing in the salivary levels of microorganisms (23, 31). There was limited data in the literature that compare the colonization of non-albicans *Candida* species in patients with fixed and removable orthodontic appliances. Our results showed that the first group has more susceptibility to colonization with no-albicans *Candida* species. Unfortunately, these species are less susceptible to common antifungal drugs than *C. albicans* (32). However, there seems to be a trend that some *C. albicans* carriers converted to non-albicans *Candida* following the insertion of the fixed appliances by unknown mechanism. This may highlight a more cautious attention concerning the possible increased risk of candidal infection, if immunosuppressed children using orthodontic treatments.

The oral cavity presents countless species of microorganisms particularly *Candida albicans*, 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 (33). Wearing complete denture like orthodontic appliances is also known as a risk factor, which can promote colonization of *Candida*, making biofilm and resulted oral candidiasis (34).

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

study confirmed that fixed appliances caused more colonization of *Candida* especially non-albicans *Candida* species. Patients using orthodontic particularly fixed appliance orthodontic therapy and their dentist should have special efforts and attention to maintain their oral hygiene and health.

#### ACKNOWLEDGEMENT

The authors would like to thank Paramedical School of Shahid Sadoughi University for providing laboratory facilities.

#### 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. *Refuat Hapeh Vehashinayim* 2013; 30: 26-30.
3. Condo R, Casaglia A, Condo SG, Cerroni L. Plaque retention on elastomeric ligatures. An in vivo study. *J Oral Implantol* 2013; 5: 92-9.
4. Dar- Odeh 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.
5. Lucas VS, Omar J, Vieira A, Roberts GJ. The relationship between odontogenic bacteraemia and orthodontic treatment procedures. *Eur J Oral Sci* 2002; 24: 293–301.
6. Brawner DL, Cutler JE. Oral *Candida albicans* isolates from non-hospitalized normal carriers, immunocompetent hospitalized patients, and immunocompromised patients with or without acquired immunodeficiency syndrome. *J Clin Microbiol* 1989; 27: 1335-1341.
7. Fotos PG, Vincent SD, Hellstein JW. Oral candidosis: Clinical, historical, and therapeutic features of 100 cases. *Oral Surg Oral Med Oral Pathol* 1992; 74: 41-49.
8. Muzyka BC. Oral fungal infections. *Dent Clin North Am* 2005; 49: 49-65.



9. Vazquez JA, Sobel JD. Mucosal candidiasis. *Infect Dis Clin North Am* 1992; 16: 793-820.
10. 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: 26-29.
11. 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.
12. Sukontapatipark W, el-Agroudi MA, Sellisetth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances: A scanning electron microscopy study. *Eur J Orthod* 2001; 23: 475-484.
13. 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: 301-308.
14. Thornberg MJ, Riolo CS, Bayirli B, Riolo ML, Van Tubergen EA, Kulbersh R. Periodontal pathogen levels in adolescents before, during, and after fixed orthodontic appliance therapy. *Am J Orthod Dentofacial Orthop* 2009 Jan; 135: 95-8.
15. Borzabadi-farahani A, Eslamipour F. Orthodontic treatment needs in an urban Iranian population, an epidemiological study of 11-14 year old children. *Euro J Pedi Dent* 2009; 10: 69-75.
16. Sandven P. Laboratory identification and sensitivity testing of yeast isolates. *Acta Odontol Scand* 1990; 48: 27-36.
17. Golia S, Mallika Reddy K, Sujatha Karjigi K, Hittinahalli V. Speciation of *Candida* using chromogenic and cornmeal agar with determination of fluconazole sensitivity. *Al Ameen J Med Sci* 2013; 6: 163-166.
18. Espinel-Ingroff A, Stockman L, Roberts G, Pincus D, Pollack J, Marler J. Comparison of Rap ID Yeast Plus System with API 20C system for identification of common, new, and emerging yeast pathogens. *J Clin Microbiol* 1998; 36: 883-886.
19. Odds FC (1988). Ecology of *Candida* and epidemiology of candidosis. In: *Candida and candidosis*. London, Philadelphia, Toronto, Sydney, Tokyo: Bailliere Tindall pp. 68-92.
20. Petti S, Barbato E, Simonetti D'Arca A. Effect of orthodontic therapy with fixed and removable appliances on oral microbiota: a six month longitudinal study. *New Microbiol* 1997; 20: 55-62.
21. Azmi M.G. Darwazeh, Al-Jasser M. The effect of fixed orthodontic appliance therapy on oral *Candida* carriage. *Saudi Dent J* 2003; 15: 141-144.
22. Alexander SA. Effects of orthodontic attachments on the gingival health of permanent second molars. *Am J Orthod Dentofacial Orthop* 1991; 100: 337-340.
23. Naranjo A A, Trivino M L, Jaramillo A, Betancourth M, Botero J E. Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. *Am J Orthod and Dentofacial Orthopedics* 2006; 30: 217-222.
24. Hagg U, Kaveewatcharanont P, Samaranayake YH, Samaranayake LP. The effect of fixed orthodontic appliances on the oral carriage of *Candida* species and Enterobacteriaceae. *Eur J Orthod* 2004; 26: 623-629.
25. Balenseifen JW, Madonia JV. Study of dental plaque in orthodontic patients. *J Dent Res* 1970; 49: 320-324.
26. Addy M, Shaw WC, Hansford P, Hopkins M. The effect of orthodontic appliances on the distribution of *Candida* and plaque in adolescents. *Br J Orthodont* 1982; 9: 157-163.
27. Mandel ID. The diagnostic use of saliva. *J Oral Pathol Med* 1990; 19: 119-25.
28. Samaranayake LP (1990). Host factors and oral candidosis. Samaranayake LP, Mac Farlane TW eds. Butterworth & Co. Ltd. pp. 75-76.
29. Sahin I, Oksuz S, Sencan I, Gulcan A, Karabay O, Gulcan E, et al. Prevalance and risk factors for yeast colonization in adult diabetic patients. *Ethiop Med J* 2005; 43: 103-9.
30. Lin AL, Johnson DA, Patterson TF, Wu Y, Lu DL, Shi Q, et al. Salivary anti-Candidal activity and saliva composition in an HIV-infected cohort. *Oral Microbiol Immunol* 2001; 16: 270-27.
31. Leung NM, Chen R, Rudney JD. Oral bacteria in plaque and invading buccal cells of young orthodontic patients. *Am J Orthod Dentofacial Orthop* 2006; 130: 11-8.
32. Krcmery V, Barnes AJ. Non-albicans *Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. *J Hosp Infect* 2002; 50: 243-60.
33. 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.