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RESEARCH ARTICLE

ORAL *CANDIDA ALBICANS* COLONIZATION RATE IN FIXED ORTHODONTICS PATIENTS

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Abstract

Objectives: The objective of this study was to assess the oral *Candida albicans* colonization (OCAC) in a cluster of teenagers and young adults while being treated with a fixed orthodontic appliance (FOA).

Subjects and methods: The investigational group was selected from orthodontic patients whom were examined clinically as soon as to get baseline information before active treatment. The cluster included 210 patients; 45 males, 165 females (mean age 21.6±4.5 years). Clinical, demographic data and risk factors were collected in standard questionnaire then each individual was directed to carry out oral wash by a phosphate-buffered saline solution, which was expectorated and processed intended for the isolation of *Candida* species on Sabouraud's dextrose agar. The isolated *Candida* species were identifying by culturing on chromogenic *Candida* agar and notice species-specific colony natures.

Results: The predominant *Candida* species isolated was *C. albicans* with OCAC rate equal to 13.8% extensively enhanced after the insertion of a FOA, as revealed by the oral rinse ($p<0.05$) techniques. The results also revealed an increase of OCAC in male patients (24.4%) than female patients (10.9%), 21-25 years patients (17.1%), and regular smoking and Qat chewing were significant associated risk factors (OR=28.6, OR=10.7 respectively, $p<0.0001$). There was no significant association between *C. albicans* colonization with oral hygiene in fixed Orthodontic patients.

Conclusion: As a whole, the current data suggest that the introduction of FOA is likely to promote OCAC. Moreover, it becomes visible that the routine oral hygiene procedures performed by these patients may not necessarily reduce OCAC while smoking and chewing Qat habits significantly increased OCAC in FOA. Also smoking and Qat chewing during FOA treatment should be banned if potential harmful effects are to be prevented. Further work with a larger sample size is required to confirm or deny these results.

Keywords: Fixed orthodontic appliance (FOA), Oral *C. albicans* colonization (OCAC); Yemen.

INTRODUCTION

The orthodontic therapy of malocclusions involves the change of mechanical energy generated from the forces of the fixed orthodontic device to a biological response in the supporting tissues and teeth and may lead to gingivitis due to regression and response to dental movement which is considered low risk as the orthodontic procedures are considered non-surgical intervention[1-4]. The microbiological flora in the oral cavity is generally a mixture of microorganisms and can include of more than 200 species[5-8]. Acid-producing bacteria are usually colonized on the surface of the teeth and around the FOA or on the orthodontic brackets which leads to enamel demineralization[8-10]. *Candida* species are most often recovered in the mouth, equal to 50% in young adults[10-13]. *Candida albicans* is the common species; on the other hand other species such as *C. parapsilosis*, *C. dubliniensis*, *C. krusei*, *C. tropicalis*, and *C. glabrata* have increased in occurrence with restricted drugs sensitive to them including allylamines, polyenes, azoles and echinocandins classes due to the development of drug resistance promptly to *Candida* species[9,10].

Furthermore, *C. albicans* found to be in 25-50% of the oral cavity of healthy persons, are one of the main

causes of microorganism biofilm formation on dentures, orthodontic appliance and catheters and isolated from about 80% of the microorganisms isolated from the oral mucosa of denture wearers[7-13].

Numerous factors, intrinsic and external, have a result on the metabolic activity, composition and pathogenicity of the highly diverse microflora of the oral cavity[3,4,9,12]. It has been reported that the existence of a FOA significantly inhibits oral hygiene and generates new retentive places for plaque and debris, which in turn affects to increased carriage of microorganisms and consequent infection[1-4]. Consequently, many have reported a relationship between an increased level of dental plaque in individuals treated with FOAs and the consequent occurrence of gingivitis[2-4]. Others researchers have revealed these topics to be more prone to periodontal disease and loss of periodontal support[1-6]. A number of clinical studies also points out an escalating occurrence of incipient carious lesions on the lingual and facial aspects of the teeth[6,7] and increased gram-positive bacterial counts in saliva[7,9] during treatment with FOAs.

The high oral colonization by the fungal pathogen *C. albicans* in individuals wearing either full or partial removable dentures is well documented[4-6]. *Candida* species have also been isolated from dental plaque and caries[4,8,13]. The aim of the present study was, therefore, to assess the oral *C. albicans* colonization (OCAC) in a group of teenagers and young adults while being treated with a fixed orthodontic appliance (FOA).

SUBJECTS AND METHODS

Subject Selection

A total of two hundred and ten people were included, during FOA treatment, who were randomly selected from Al-Thawra Hospital, Al-Gomhoria Hospital, Faculty of dentistry Sana'a University clinics and Dental Centers in Sana'a City, Yemen. The duration of the study was six months period, started in August 2019 and ended in February 2020. Inclusion criteria for subject selection were healthy individuals with no clinical signs of *Candida* infection and no systemic disease. In addition, individuals who currently taking antifungal, steroids, antibiotics, or immunosuppressive drugs in the past 6 months were excluded.

Collection and identification of samples: Saliva samples were collected using the oral rinse technique[14]. In summary, each subject was required to rinse the mouth for 60 seconds using 10 ml of a phosphate sterile saline (PBS, 0.01 M phosphate buffered saline, pH 7.2) and eject the rinse into a sterile 15 ml container[15]. The samples were immediately transported on ice to the microbiology laboratory. Each oral rinse was centrifuged at 3500 rpm for 10 minutes, and then the supernatant was discarded. The pellet was re-suspended in 1ml sterile PBS. One hundred µl of the concentrated oral rinse was inoculated onto Sabouraud's dextrose agar and incubated at 37°C for 48 hours. The lasting samples were stored at -20°C. If *Candida* colonies appeared on the Sabouraud's dextrose agar, then chromogenic *Candida* agar was inoculated using 100 µl of the oral rinse supernatant and incubated for 48 hours for colonies study. *Candida* species were identified by the color of the colonies using the color reference guide supplied by the manufacturer. When color identification was unclear, fermentation assay of sucrose, maltose, glucose, lactose and galactose was done. The *Candida* species were also identified by the ability to produce chlamydo-spores on glutinous rice agar[16].

Data analysis

The data was statistically analyzed using EPI-Info version 6. The difference in the distribution of *C. albicans among* groups was based on a comparison of frequency distributions by chi-square test. The value of $p<0.05$ was considered significant.

Ethical approval

We obtained written consent in all cases. Approval was obtained from the participants prior to collection of samples. The study proposal was evaluated and approved by the Ethics Committee, Faculty of Medicine and Health Sciences, University of Sana'a.

RESULTS

Table 1 shows the age and gender distribution of patients with fixed orthodontics at a selected dental clinic in Sana'a. 78.6% of the participants are female and only 21.4% are male. The age average ± SD for participants was 21.6±4.5 years. Most of the subjects covered were in the age group 21-25 years (55.7%) followed by 16-20 years (29%). Table 2 shows the distribution of different types of *Candida* species among Fixed Orthodontic patients. The predominant isolated *Candida* species were *C. albicans* with a significantly improved OCAC rate of 13.8% after the introduction of FOA. Also others species were isolated in which *C. glabrata* isolated from 3 patients, *C. tropicalis* from 3 patients, and *C. parapsilosis* isolated from 1 patients.

Table 1: The age and sex distribution of patients with fixed orthodontics at a selected dental clinic in the city of Sana'a.

Characters	Number	Percentage
Sex		
Male	45	21.4
female	165	78.6
Age groups		
≤15 years	12	5.7
16-20 years	61	29
21-25 years	117	55.7
>25 years	20	9.5
Total	210	100
Mean age	21.6 years	
SD	4.5 years	
Median	21 years	
Mode	21 years	
Min	13 years	
Max	25 years	

On the other hand, two cases had a combined infection with of *C. albicans+ C. glabrata* and two cases with *C. albicans+ C. tropicalis*. The results also revealed an increase in OCAC in male patients (24.4%) than

female patients (10.9%), 21-25 years old patients (17.1%), (Table 3) and regular smoking and chewing Qat were important associated risk factors (OR=28.6, OR=10.7, respectively) (Table 4). On the other hand there was no significant association between colonization of *C. albicans* with the application of different oral hygiene practices in fixed orthodontic patients (Table 5).

Table 2: Distribution of different types of *Candida* species among fixed orthodontic patients.

***Candida* species**	**Number**	**Percentage**
Candida albicans	25	11.9
Candida glabrata	3	1.4
Candida tropicalis	3	1.4
Candida parapsilosis	1	0.5
Candida albicans+ Candida glabrata	2	1
Candida albicans+ Candida tropicalis	2	1
Total *C. albicans*	29	13.8
Total Candida species	36	17.14

DISCUSSION

The current study, explored OCAC rate through fixed orthodontic therapy, indicates that the wearing of such appliances leads to enhanced carriage and extensive changes in the oral microorganism population, probably due to the appliance-induced ecological alterations within the oral cavity. The OCAC primary absence of the baseline patient cluster was not unexpected, as applicants were requested to establish good oral hygiene prior to the trial. However, after the introduction of FOA, a 13.8% increase in the OCAC rate was observed in the test group. The incidence of orthodontic attachments on the labial and lingual surfaces of these teeth is likely to be the cause for this observation, as they interfere with thorough brushing of the gingival area. Similar changes in OCAC rate during orthodontic treatment with removable and fixed appliances have been reported by several authors[1,2,8,9]. Furthermore, the presence of rough-surfaced bonding material in FOA or dentures acting as a *C. albicans* trap and a gingival irritation[4,6,8,9-13] may have played a causative role. Thus, a significant increase in the OCAC rate after the introduction of FOA in the current study may be partly due to the patient's attitude and behaviour, in addition to the presence of FOA which made it difficult to maintain dental hygiene. Thus, although the orthodontic device may have a detrimental effect on plaque control, this may be reduced through regular advice and instructions, which may have a lasting effect. Also, it may be assumed that foreign substances, including appliances or dental prostheses, change the oral natural environment by mechanisms at present unidentified, such that the propagation of micro-organisms.

On the other hand, number of researchers[9-13,15-17] have revealed that the existence of a prosthesis or an appliance enhances candidal numbers. Arendorf and Addy[6] inspected 33 individuals who experience removable orthodontic appliance therapy and discovered a direct connection between the occurrence of a removable orthodontic appliance and oral *Candida* species colonization. Of the *Candida* species isolated in the current study, the most predominant was *C. albicans*, while *C. glabrata*, *C. tropicalis* and *C. parapsilosis* were isolated less frequently. This supports the finding that *C. albicans* is the only most prevalent *Candida* species in the oral cavity[9-13,17].

Table 3: Distribution of *C. albicans* in relation to gender and age among fixed orthodontic patients.

Characters	**Positive *C. albicans*, n=29**		**OR**	**CI**	**X^2**	***p***
	No	**%**				
Sex						
Male, n=45	11	24.4	2.6	1.14-6.1	5.4	0.01
Female, n=165	18	10.9	0.37	0.1-0.8	5.4	0.01
Age groups						
≤15 years, n=12	1	8.3	0.55	0.08-4.8	0.33	0.57
16-20 years, n=61	7	11.5	0.74	0.3-1.8	0.39	0.5
21-25 years, n=117	20	17.1	1.9	0.83-4.1	2.3	0.1
>20 years, n=20	1	5	0.3	0.04-2.5	1.4	0.22
Total n=210	29	13.8				

Table 4: Correlation of *Candida* species colonization with the habits of fixed orthodontic patients.

Habits	**Positive *C. albicans***		**OR**	**CI**	**X^2**	***p***
	No	**%**				
Regular smoking						
Yes n=18	13	72.2	28.6	9-90	56	<0.0001
No n= 192	16	8.3	0.03	0.01-0.1	56	<0.0001
Regular Qat chewing						
Yes n=42	18	42.8	10.7	4.5-25.4	37.1	<0.0001
No n= 168	11	6.5	0.09	0.03-0.2	37.1	<0.0001
Regular Shamahe						
Yes n=4	1	25	2.1	0.2-21	0.4	0.51
N0 n= 206	28	13.6	0.47	0.04-4.6	0.4	0.51

Table 5: Correlation of *Candida* species colonization with oral hygiene for fixed orthodontic patients.

Oral hygiene	Positive *Candida albicans*, n=29		OR	CI	X^2	*p*
	No	%				
Regular tooth brush						
Yes n=205	28	13.6	0.6	0.06-5.8	0.16	0.68
No n= 5	1	20	1.5	0.17-14.9	0.16	0.68
Regular Rinse						
Yes n=31	12	38.7	0.6	0.06-5.8	0.16	0.68
No n= 179	17	9.5	1.5	0.17-14.9	0.16	0.68
Regular Flossing						
Yes n=16	3	6.25	1.4	0.3-5.5	0.35	0.55
N0 n=194	26	13.4	0.67	0.17-2.5	0.35	0.55

The records also prove preceding results that more variant *Candida* species can be isolated by means of the oral rinse technique than the imprint culture or pooled plaque technique[9-13,17]. Also, the predominance of *C. albicans* can be explained by the fact that *C. albicans* are microorganisms with an elevated adhesion capacity to the oral mucous. This adherence is enhanced *in vitro* when *Candida* is incubated simultaneously with *S. mutans* (*S. mutans*), *S. sanguis*, *S. salivairus* or some other bacteria[18]. Also its predominant rise from that it is an only one of its type parasite able of colonizing, infecting, and continuing on mucosal surfaces, and motivating mucosal immune responses[10,11]. Attack of tissues by *C. albicans* is supported by hyphal growth. The alteration of budding *C. albicans* to hyphal growth is endorsed by physical connection with surfaces and is underneath genetic control. After *C. albicans* colonize an epithelial or epidermal surface, they stick to host cells and create depressions in the surface of host cells. As *C. albicans* -form cells modify to the hyphal form, these hyphae are able to diffuse into the surface of the tissue layer. The route of hyphal growth is established by the topography of the substratum. Hyphae are directed by ridges in the tissue layer; this manners is identified as thigmotropism[8-13,18].

The results of the current study revealed an increase in OCAC in male patients (24.4%) than female patients (10.9%). The present study results supported the rejection of the null hypothesis which states that there would be no difference between male and female FOA in terms of the prevalence of OCAC and colonization by *C. albicans* of the surfaces of fixed orthodontic appliance and attachment surroundings. Regular smoking and chewing Qat were significant risk factors for OCAC in FOA patients in the present study (OR = 28.6, OR=10.7, respectively, $p<0.0001$) (Table 4). Result of current study is similar to that reported by Tarcin in which a high significant risk of colonization was associated with smoking habit[19]. This result can be explained by the fact that smoking, especially heavy smoking, is a predisposing factor for OCAC but the reasons for this relationship is unknown. One hypothesis is that cigarette smoke contains nutritional factors for *C. albicans*, or local epithelial changes that help colonize *Candida* types and smoking kill immune cells and damage the mucous membrane[9,10,19,20]. There was no effect for mouth hygiene in occurring of colonization of *C. albicans* among current study subjects. This result is different from that reported by several studies[5,6,17,21] in which a high significant risk of mouth colonization was associated with bad mouth hygiene.

CONCLUSIONS

Treatment with a FOA may change the ecology in the mouth by introducing new stagnant parts available for colonization and maintenance of *C. albicans* and other *Candida* species. The outcomes show this by indicating that FOAs have a direct result upon the occurrence and concentration of *Candidal* carriage in this group of adolescents and young adults. The appliances may also interfere with oral hygiene practice as FOAs cover extensive parts of the tooth surfaces with metal and composite materials.

In clinical terms, these results indicate that regular advice and routine instruction in oral and appliance hygiene given to this group of patients did not overcome totally the possible unfavorable effects of OCAC. But rregular smoking and chewing Qat direct effect the OCAC in FOAs patients. Therefore, specific awareness has to be paid to the OCAC control of patients undertake FOA therapy, also smoking and Qat chewing during FOA treatment should be banned if potential harmful effects are to be prevented.

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AUTHOR'S CONTRIBUTION

This research work is part of a Master's thesis. **Saleh AL-amri MA:** conducted field works, laboratory works and wrote up the thesis. **Shoga Al-deen HM:** writing, review and editing. **Ahmed Ali O:** methodology, investigation. **Al-Shamahy HA:** editing, supervision. **Al-Shami IZ:** data curation, conceptualization. **Al-labani MAC:** methodology, formal analysis.

DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request. All authors revised the article and approved the final version.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. Kaveewatcharanont Hägg P, Samaranayake YH, Samaranayake LP. The effect of fixed orthodontic appliances on the oral carriage of *Candida* species and Enterobacteriaceae. European J Orth 2004; 26:623–629. *https://doi.org/10.1093/ejo/26.6.623*
2. Atack N E, Sandy J R, Addy M. Periodontal and microbiological changes associated with the placement of orthodontic appliances: a review. J Periodontol 1996; 67: 78–85. *https://doi.org/10.1902/jop.1996.67.2.78*
3. Dar-Odeh Najla, Shehabi Asem, Al-Bitar Zaid, *et al*. Oral *Candida* colonization in patients with fixed orthodontic appliances: The importance of some nutritional and salivary factors. African J Micro Res 2011; 5(15):2150-2154. *https://doi.org/10.5897/AJMR11.382*
4. 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 Clinl Exp Dent 2013; 5(1): e36–e41. *https://doi.org/10.4317/jced.50988*
5. Brusca MI, Chara O, Sterin-Borda L, Rosa AC. Influence of different orthodontic brackets on adherence of microorganisms *in vitro*. Angle Orthodontist 2007: 77(2): 331–336.*https://doi.org/10.2319/0003-3219(2007)077[0331:IODOBO]2.0.CO;2*
6. Arendorf T, Addy M. Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. J Clinl Periodontol 1985; 12(5): 360–368.*https://doi.org/10.1111/j.1600-051x.1985.tb00926.x*
7. Wilson M. Bacterial biofilms and human disease. Science Progress 2001; 84(3): 235–254.
8. Hibino K, Wong RW, Hagg U, Samaranayake LP. The effects of orthodontic appliances on the human mouth. Int J Paed Dent 2009; 19:308. *https://doi.org/10.1111/j.1365-263X.2009.00988.x*
9. Al-Kebsi AM, Othman MO, AlShamahy HA, *et al*. Oral c.albicans colonization and non-*Candida albicans* candida colonization among university students, Yemen. Universal J Pharm Res 2017; 2(5):5-11. *https://doi.org/10.22270/ujpr.v2i5.R2*
10. Al-Sanabani NF, Al-Kebsi AM, Al-Shamahy HA, Abbas AKM. Etiology and risk factors of stomatitis among Yemeni denture wearers. Universal J Pharm Res 2018; 3 (1): 69–73. *https://doi.org/10.22270/ujpr.v3i1.R9*
11. Al-Shamahy HA, Abbas AMA, Mahdie Mohammed AM, Alsameai AM. Bacterial and fungal oral infections among Patients Attending Dental Clinics in Sana'a City-Yemen. On J Dent Oral Health 2018; 1(1): 1-8. *https://doi.org/10.26717/BJSTR.2018.11.002072*
12. Al-Dossary OAE, Hassan A Al-Shamahy. Oral *Candida albicans* colonization in dental prosthesis patients and individuals with natural teeth, Sana'a city, Yemen. Biomed J Sci Tech Res 2018; 11(2):1-7. *https://doi.org/10.26717/BJSTR.2018.11.002072*
13. Al-Haddad KA, Al-dossary OAE, Al-Shamahy HA. Prevalence and associated factors of oral non-*Candida albicans* Candida carriage in denture wearers in Sana'a city-Yemen. Universal J Pharm Res 2018; 3(4): 7-11. *https://doi.org/10.22270/ujpr.v3i4.176*
14. Coulter WA, Kinirons MJ, Murray SD. The use of a concentrated oral rinse culture technique to sample oral *candida* and *lactobacilli* in children and the relationship between Candida and Lactobacilli levels and dental caries experience: A pilot study. Int J Paediatr Dent 1993; 3(1): 17- 21. *https://doi.org/10.1111/j.1365-263x.1993.tb00042.x*
15. MacFarlane TW, Samaranayake LP, Williamson MI. Comparison of Sabouraud dextrose and Pagano-Levin agar media for detection and isolation of yeasts from oral samples. J Clin Microbiol 1987; 25(1): 162-164. 18. PMID: 3539988.
16. Staib P, Morschhäuser J. Chlamydospore formation in *Candida albicans* and *Candida dubliniensis* - an enigmatic developmental programme. Mycoses 2007; 50(1): 1-12. *https://doi.org/10.1111/j.1439-0507.2006.01308.x*
17. W, Al-Saigh RJ, Al-Dabagh NN, *et al*. Oral *Candida* in patients with fixed orthodontic appliance: *in vitro* combination therapy. Bio Med Res Int 2017; 2017: 8-16. *https://doi.org/10.1155/2017/1802875*
18. Greenberg MS, Glick M, Ship JA. Burket's oral medicine (11th ed. ed.). Hamilton, Ont.: BC Decker. 2008; 79–84.
19. Tarçin, BG. Oral candidiasis: etiology, clinical manifestations, diagnosis and management. MÜSBED. 2011; 1(2):140-148. PMID:9573835
20. Kumaraswamy KL, Vidhya M Rao PK, Mukunda A. Oral biopsy: oral pathologist's perspective. J Cancer Res Therap 2012; 8(2): 192-8. *https://doi.org/10.4103/0973-1482.98969*
21. Rautemaa R, Ramage G. Oral candidosis- clinical challenges of a biofilm disease. Crit Rev Microbiol 2011; 37 (4): 328-36. *https://doi.org/10.3109/1040841X.2011.585606*