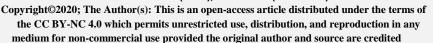


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#### **RESEARCH ARTICLE**

## ORAL CANDIDA ALBICANS COLONIZATION RATE IN FIXED ORTHODONTICS PATIENTS

Hussein Mohammad Shoga al-deen<sup>1</sup>, Ahmed Ali Obeyah<sup>1</sup>, Hassan Abdulwahab Al-Shamahy<sup>2</sup>
Ibrahim Zaid Al-Shami<sup>3</sup>, Manal Ahmad Saleh AL-amri<sup>2</sup>, Mohammed A Al-labani<sup>1</sup>

<sup>1</sup>Orthodontics, Pedodontics and Prevention Department Faculty of Dentistry, Sana'a University, Yemen.

<sup>2</sup>Department of Basic Sciences, Faculty of Dentistry, Sana'a University, Republic of Yemen.

<sup>3</sup>Department of Conservative Dentistry and Oral Health, Faculty of Dentistry, Sana'a University, Yemen.

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#### \*Address for Correspondence:

**Dr. Hassan A. Al-Shamahy**, Faculty of Dentistry, Sana'a University, P.O. Box 775 Sana'a, Yemen, Tel: +967-1-239551. E-mail: shmahe@yemen.net.ve

### 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<sup>1-4</sup>. The microbiological flora in the oral cavity is generally a mixture of microorganisms and can include of more than 200 species<sup>5-8</sup>. 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<sup>8-10</sup>. 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 occurrence with restricted drugs sensitive to them including allylamines, polyenes, azoles echinocandins classes due to the development of drug resistance promptly to Candida species<sup>9,10</sup>.

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

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dentures, orthodontic appliance and catheters and isolated from about 80% of the microorganisms isolated from the oral mucosa of denture wearers<sup>7-13</sup>. Numerous factors, intrinsic and external, have a result the metabolic activity, composition pathogenicity of the highly diverse microflora of the oral cavity<sup>3,4,9,12</sup>. 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 infection<sup>1-4</sup>. microorganisms and consequent Consequently, many have reported a relationship between an increased level of dental plaque in individuals treated with FOAs and the consequent occurrence of gingivitis<sup>2-4</sup>. Others researchers have revealed these topics to be more prone to periodontal disease and loss of periodontal support<sup>1-6</sup>. A number of clinical studies also points out an escalating occurrence of incipient carious lesions on the lingual and facial aspects of the teeth<sup>6,7</sup> and increased gram-positive bacterial counts in saliva<sup>7,9</sup> during treatment with FOAs.

causes of microorganism biofilm formation on

The high oral colonization by the fungal pathogen *C. albicans* in individuals wearing either full or partial removable dentures is well documented<sup>4-6</sup>. *Candida* species have also been isolated from dental plaque and caries<sup>4,8,13</sup>. 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<sup>14</sup>. 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<sup>15</sup>. 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 resuspended in 1ml sterile PBS. One hundred ul 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 chlamydospores on glutinous rice agar<sup>16</sup>.

#### **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.

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			

#### 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. 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.

species among fixed of thoughtie 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+	2	1				
Candida glabrata						
Candida albicans+	2	1				
Candida tropicalis						
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<sup>1,2,8,9</sup>. Furthermore, the presence of rough-surfaced bonding material in FOA or dentures acting as a C. albicans trap and a gingival irritation<sup>4,6,8,9-13</sup> 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<sup>9-13,15-17</sup> have revealed that the existence of a prosthesis or an appliance enhances candidal numbers. Arendorf and Addy<sup>6</sup> 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<sup>9-13,17</sup>.

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	%	<del>_</del>			
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 <sup>2</sup>	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

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

Oral hygiene	Positive Candida albicans, n=29		OR	CI	X <sup>2</sup>	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 Streptococcus mutans (S. mutans), Streptococcus sanguis, Streptococcus salivairus or some other bacteria<sup>18</sup>. 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 thigmotropism8-13,18.

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<sup>19</sup>. 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<sup>5</sup>,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.

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#### CONFLICT OF INTEREST

None to declare.

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