



Research Article

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Oral Non-Candida Albicans Species Colonization rate in Fixed Orthodontics Patients

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Abstract

Objectives: The aim of this investigation was to assess the oral Non-*Candida albicans* colonization (ONCAC) in a group of teenagers and young adults while being treated with a fixed orthodontic appliance (FOA).

Subjects and methods: The experimental group was selected from a sample of orthodontic patients who were clinically examined once to obtain baseline data before active treatment. The group comprised 210 subjects; 45 males, 165 females (mean age 21.6 ± 4.5 years). Clinical, demographic data and risk factors were collected in standard questionnaire then each subject was directed to carry out oral rinsing using a phosphate-buffered saline solution, which was expectorated and processed for the recovery of *Candida* species on Sabouraud's dextrose agar. Isolates were identifying by culturing on chromogenic *Candida* agar and noting species-specific colony characteristics

Results: The overall rate of *Candida* species oral colonization in FOA was 17.1%. The predominant Non-*Candida* species isolated was *C. tropicalis* and *Candida glabrata* with total ONCAC rate equal to 5.2% significantly increased after the insertion of a FOA, as detected by the oral rinse ($P < 0.05$) techniques. The results also revealed an increase of ONCAC in male patients (8.9%) than female patients (4.2%), ≤ 15 years patients (8.3%), and 16-21 years (8.2%) and regular smoking was significant associated risk factor (OR=7.5, 95% CI=1.9-28.9, $P = 0.0007$). There was no significant correlation between ONCAC with oral hygiene in fixed Orthodontic patients, while negative effects were found for regular rinsing (colonization=12.9%, OR= 3.6, $p = 0.03$).

Conclusion: Taken together, these data suggest that the introduction of FOA is likely to promote ONCAC. Moreover, it appears that the routine oral hygiene procedures performed by these patients may not necessarily reduce ONCAC while smoking habits significantly increased ONCAC in FOA. Also smoking 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: Oral Non-*Candida albicans* colonization (ONCAC); fixed orthodontic appliance (FOA); Yemen

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Introduction:

More than 40 types of *candida* yeast, the most common being *Candida albicans*, can cause infection in humans. In addition, *C. tropicalis*, *C. parapsilosis*, and *C. glabrata* are pathogenic species of major *Candida* that are collectively referred to as Non-*Candida albicans* species [1-7]. Many types of *candida* species colonize mucous surfaces in the oral cavity, digestive system and vagina. *Candida* is a common natural organism in the mouth and does not cause any problems in healthy people. In the general population, reports indicate that carriage rates are reported to be in the range 3 to 75% without any symptoms [8]. However, the overgrowth of *Candida* in the pharyngeal or esophageal mucosa causes a burning sensation, taste disturbances, severe *mucositis*, or *dysphagia* and leads to malnutrition [8]. Once the oral cavity is colonized, *Candida* species become more accessible to the respiratory system, and since they are a homogeneous type of intestinal cavity and skin surfaces, the colonization index increases [9,10]. Consequently, oral *candida* colonization poses a risk of systemic disorders as well as local mucosal infections in patients with FOA treatment. Previous studies indicate that the risk factors

involved in oral *Candida* colonization in individuals include dentures wear, FOA, poor oral hygiene / dentures / FOA, and low local salivation flow [11-17]. It has been observed that the presence of a FOA may significantly prevent oral hygiene and generate places where food remains, which in turn affects an increase in the reproduction and carriage of microorganisms; and the consequent infection [14-17]. Orthopedic treatment for malocclusion involves converting mechanical energy generated from the forces of the fixed orthodontic device (FOA) to a biological reaction in the teeth and supporting tissues and may lead to gingivitis due to slope and response to dental movement which is considered low risk as orthodontic procedures are considered non-surgical intervention [13-17]. Hence, the threats of ONCAC should be elucidated in conditions of both systemic and oral conditions, especially in people with FOA who are more probable to develop *Candidiasis* as well as *candidial stomatitis*. The objective of this study, therefore, was to assess ONCAC in a group of adolescents and young adults whilst being treated with a FOA.

Subjects and Laboratory Methods:

Subject Selection:

A two hundred and ten people were studied, in the course of FOA treatment, as they were randomly selected from, Al-Jumhoria Hospital, Al-Thawra Hospital, University of Sana'a College of Dental Clinics and Dental Centers in Sana'a, Yemen. The duration of the study was six months, it taking place in August 2019 to February 2020. The inclusion criteria for subject choice were healthy individuals with no clinical signs of candidiasis and no systemic disease. Additionally, subjects who have presently taken steroids, antifungals, antibiotics or immunosuppressive drugs in the past six months have been excluded.

Collection and identification of samples:

Saliva specimens were gathered by means of the mouth rinse method [18]. In brief, each subject was requisited to rinse the mouth for 60 seconds with 10 mL sterile phosphate saline (PBS, 0.01M phosphate dilute brine, pH 7.2) and rinsed out in a sterile 15 mL container [19]. After that, the samples were transferred on ice to the microbiology laboratory where each oral rinse was centrifuged at 3,500 rpm

for 10 minutes, and the supernatant material was eliminated. Pellet was resuspended in sterile 1 ml PBS. One hundred μ l of mouth rinse (concentrated), was inoculated onto Sabouraud's dextrose agar and incubated at 37 ° C for 48 hours. If *Candida* colonies appear in the Sabouraud's dextrose agar, then chromogenic *Candida* agar was inoculated using 100 μ l of the oral rinse supernatant and incubated for 48 hours to study the colonies. *Candida* species were determined by the color of the colonies using the manufacturer's reference color guide. When the color determination was unclear, a fermentation test was performed for maltose, sucrose, glucose, galactose and lactose. *Candida* species have also been identified through the ability to produce chlamydia spores in glutinous rice agar [20].

Data Analysis:

The data was statistically analyzed using EPI-Info version 6. The difference in the distribution of Non-*Candida 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 \pm 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% while ONCAC was 5.2% after the introduction of FOA. ONCAC species were *Candida glabrata* isolated from 3 patients, *Candida tropicalis* from 3 patients, and *Candida parapsilosis* isolated from 1 patients.

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	

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

<i>Candida species</i>	Number	Percentage
<i>Candida albicans</i> only	25	11.9
<i>Candida glabrata</i> only	3	1.4
<i>Candida tropicalis</i> only	3	1.4
<i>Candida parapsilosis</i> only	1	0.48
<i>Candida albicans</i> + <i>Candida glabrata</i>	2	0.95
<i>Candida albicans</i> + <i>Candida tropicalis</i>	2	0.95
Total <i>Candida albicans</i>	29	13.8
Total non- <i>Candida albicans</i>	11	5.2
Total <i>Candida species</i> colonization	36	17.1

Table 2: Distribution of different types of *Candida species* among Fixed Orthodontic patients.

Characters	Positive ONCAC n=11		OR	CI	X ²	P
	No	%				
Sex						
Male n=45	4	8.9	2.2	0.6-7.8	1.5	0.2
Female n=165	7	4.2	0.45	0.12-1.6	1.5	0.2
Age groups						
≤15 years N=12	1	8.3	1.7	0.2-4.5	0.24	0.62
16-20 years N=61	5	8.2	2.1	0.6-7.2	1.5	0.2
21-25 years N=117	4	3.4	0.43	0.1-1.5	1.7	0.18
>20 years n=20	1	5	0.94	0.11-7.8	0.002	0.95
Total n=210	11	5.2				

Oral Non-*Candida albicans* colonization =ONCAC ,Odds ratio =OR , X² =Chi square test, p=p value

Table 3: Distribution of ONCAC in relation to gender and age among Fixed Orthodontic patients

Habits	Positive ONCAC n=11		OR	CI	X ²	P
	No	%				
Regular smoking						
Yes n=18	4	22.2	7.5	1.9-28.9	11.4	0.0007
No n= 192	7	3.64	0.13	0.03-0.5	11.4	0.0007
Regular Qat chewing						
Yes n=42	4	9.5	2.4	0.6-8.6	1.9	0.16
No n= 168	7	4.2	0.41	0.11-1.1	1.9	0.16
Regular Shamahe						
Yes n=4	1	25	6.5	0.1-68	3.2	0.07
No n= 206	10	4.85	0.15	0.01-1.6	3.2	0.07

Oral Non-Candida albicans colonization =ONCAC ,Odds ratio =OR , X² =Chi square test, p=p value

Table 4: Correlation of ONCAC with the habits of Fixed Orthodontic patients

Oral hygiene	Positive ONCAC n=11		OR	CI	X ²	P
	No	%				
Regular tooth brush						
Yes n=205	10	4.9	0.2	0.02-2	2.2	0.113
No n= 5	1	20	4.8	0.4-47	2.2	0.113
Regular Rinse						
Yes n=31	4	12.9	3.6	1.0-13	4.3	0.03
No n= 179	7	3.9	0.27	0.07-1.0	4.30	0.03
Regular Flossing						
Yes n=16	1	6.25	1.2	0.1-10.2	0.035	0.8
No n=194	10	5.2	0.81	0.09-6.8	0.035	0.8

Table 5: Correlation of ONCAC with Oral Hygiene for Fixed Orthodontic patients

On the other hand, two cases had a combined infection with of *Candida albicans*+ *Candida glabrata* and two cases with *Candida albicans*+ *Candida tropicalis*. The results also revealed an increase of ONCAC in male patients (8.9%) than female patients (4.2%), ≤ 15 years patients (8.3%), and 16-21 years (8.2%) (table 3) and regular smoking was significant associated risk factor (OR=7.5, 95% CI=1.9-28.9, P= 0.0007) (table 4). There was no significant correlation between ONCAC with oral hygiene in fixed Orthodontic patients while negative effects were found for regular rinsing where the ONCAC rate rises to 12.9%, with OR = 3.6, CI = 1-13, p = 0.03 in people performing regular rinsing (table 5).

Discussion:

There have been numerous reports of the prevalence of *Candida* types in denture wearers patients [3-7]. However, information regarding ONCAC rates is limited. This study, which investigated the ONCAC rate for the period of fixed orthodontic treatment, indicates that wearing these devices leads to increased carriage and significant changes in the number of oral microorganisms, perhaps due to environmental changes caused by the device within the oral cavity [13-16]. The sampling method for *Candida* culture was different from other studies exploring the same problem. In this study, saliva specimens were collected by the mouth rinse method [18], where the samples were collected and cultured, and unlike another study, more than one technique was used [21]. In the present study, the total rates of *Candida* proliferation and ONCAC acquired using the mouth rinse technique illustrate an increase in oral colonization of candida species after the introduction of a FOA.

The primary absence of ONCAC for the baseline patient group was not surprising, as participants were asked to establish good pre-trial oral hygiene. Nevertheless, after introducing FOA, a 5.2% raise in the ONCAC rate was observed in the study group. The occurrence of orthodontic attachments on the labial and lingual surfaces of these teeth may be the reason for this observation, as they interfere with careful cleaning of the gingival area. Comparable changes in the ONCAC rate during orthodontic treatment with removable and fixed devices have been reported by many authors [13, 14, 10]. Moreover, the presence of rough surface bonding materials in the FOA or dentures serving as a *candida* species trap and a gingival irritation [16, 13-17] may have played a causative role. Therefore, the significant increase in the ONCAC rate after the induction of FOA in the this study may be in part due to the patient's manner and behavior, as well to the presence of the FOA which made it difficult to maintain dental hygiene. Moreover, it may be assumed that foreign bodies, including dental prostheses or appliances, alter the oral environment with mechanisms that are not yet known, such as encouraging the proliferation of organisms, such as encouraging the proliferation of non-*Candida albicans* species.

There is in spite of this, no realistic indication that FOA insertion will alter a non-carrier state into a carrier state. Longer-term researches are essential to test this hypothesis. Alternatively, number of researchers [3-7, 12, 20] have revealed that the existence of a prosthesis or an appliance increases *candidal* numbers, not only at the occluded place, but at all mucosal sites sampled.

The aim of this study was to investigate oral *Candida* colonization in individuals provided with FOA in connection with important factors specifically: sex, age, smoking, Qat chewing, and oral hygiene. It is noted that in many studies [13-17], older age groups were more susceptible than younger age groups but there were no significant variations between younger and older age groups in the ONCAC rate in our study. This lack of correlation in our patients may be attributed to the fact

that the immune responses in this group of healthy young individuals were well developed, also, no other study was able to detect this correlation, although the study groups were institutionalized elderly [22].

Results of the current study showed an increase in ONCAC in male patients (8.9%) than female patients (4.2%). The results of the current study supported the rejection of the null hypothesis which states that there will be no difference between FOA for male and female in terms of ONCAC prevalence and colonization by non-*C. albicans* species of fixed orthodontic surfaces and their attachment surroundings.

Regular smoking was significant risk factor for ONCAC in FOA patients in the present study (OR = 7.5, p=0.0007) (Table 4). Our result is comparable to that mentioned by Tarcin [23] in which a significant risk of colonialism was associated with the smoking habit. This result can be explained by the fact that smoking, especially heavy smoking, is a predisposing factor for ONCAC but the reasons for this relationship are unknown.

One hypothesis is that cigarette smoke contains nutritional factors for *Candida* species, or local epithelial changes that help colonize *Candida* types and smoking kill immune cells and damage the mucous membrane [3,4,23,24]. While according to some other researchers tobacco smoking is not related to oral *candidiasis* [25]. There was no effect for mouth hygiene in occurring of ONCAC among current study subjects. This result is different from that reported by several studies [17,26-28] in which a high significant risk of mouth colonization of *Candida* species was associated with bad mouth hygiene.

Conclusions:

In spite of the limitations of this study, it can be concluded that the provision of FAO enhances ONCAC. The FOA may also interfere with practice of oral hygiene as they cover considerable parts of the tooth surfaces with composite materials and metal. In addition regular smoking direct effect the ONCAC in FOAs patients, Hence, particular attention has to be paid to the ONCAC control of patients undergoing FOA therapy, also smoking during FOA treatment should be banned if potential harmful effects are to be prevented.

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Conflict of Interest :

"No conflict of interest associated with this work".

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