Original Research Article

ORAL *CANDIDA ALBICANS* COLONIZATION RATE IN FIXED ORTHODONTICS PATIENTS

ABSTRACT

Objectives: The aim of this investigation was to assess the oral *Candida albicans* colonization (OCAC) 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 predominant *Candida* species isolated was *C. albicans* with OCAC rate equal to 13.8% 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 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 correlation between *C.albicans* colonization with oral hygiene in fixed Orthodontic patients.

Conclusion: Taken together, these data suggest that the introduction of FOA is likely to promote OCAC. Moreover, it appears 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 Qhat 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: : Oral *C.albicans* colonization (OCAC); fixed orthodontic appliance (FOA); Yemen

INTRODUCTION

The orthodontic treatment of malocclusions includes the conversion of 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 regression and response to dental movement which is considered low risk as the orthodontic procedures are considered non-surgical intervention[1-4]. The microbial flora in the mouth are usually a mixture of microorganisms and can consist 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 found in the oral cavity, with up to 50% in young adults [10-13]. *Candida albicans* is the prevalent species; on the other hand other species such as *C. parapsilosis, C. dubliniensis, C. krusei , C. tropicalis*, and *C. glabrata* have raised in frequency with limited antifungal drugs sensitive to them including polyenes, azoles, allylamines, and echinocandins classes due to the evolution of drug resistance rapidly to *Candida* species [9,10]. Furthermore, *candida 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].

Many factors, intrinsic and external, have an effect on the composition, metabolic activity, and pathogenicity of the highly diverse microflora of the oral cavity [3,4,9,12]. It has been reported that the existence of a fixed orthodontic appliance (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 increasing incidence 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 *Candida 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 *Candida albicans* colonization (OCAC) in a group of teenagers and young adults while being treated with a fixed orthodontic appliance (FOA).

SUBJECTS AND LABORATORY 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, 0.01M 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 ^{o}C for 48 hours. The lasting samples were stored at -20^{o} C. If *Candida* colonies appeared on the Sabouraud's dextrose agar, then chromogenic *Candida* agar was inoculated using 100µl 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 *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 ± 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 *Candida glabrata* isolated from 3patients, *Candida tropicalis* from 3 patients, and *Candida parapsilosis* isolated from 1 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 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).

DISCUSSION

This study, which explored OCAC rate during fixed orthodontic therapy, indicates that the wearing of such appliances leads to increased carriage and considerable changes in the oral microorganism population, possibly due to the appliance-induced ecological alterations within the oral cavity. The OCAC primary absence of the baseline patient group was not surprising, as participants were required to establish good oral hygiene prior to the trial. However, after the introduction of FOA, a 13.8 percent 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 *candida 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 behavior, 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 postulated that foreign objects, including dental prostheses or appliances, alter the oral environment by mechanisms as yet unknown, such that the proliferation of organisms, such as *Candida* species, is encouraged. There is, however, no convincing evidence that FOA insertion will change a non-carrier state into a carrier state. Longer-term investigations are necessary to test this hypothesis. On the other hand, number of researchers[9-13, 15-17] have shown

that the existence of a prosthesis or an appliance increases *candidal* numbers, not only at the occluded site, but at all mucosal sites sampled. Arendorf and Addy [6] investigated 33 patients who underwent removable orthodontic appliance therapy and found a direct relationship between the presence 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 *Candida glabrata , Candida tropicalis* and *Candida parapsilosis* were isolated less frequently. This supports the finding that *C. albicans* is the single most predominant *candidal* species in the oral cavity [9-13,17]. The data also confirm previous findings that more variant *Candida* species can be isolated using 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 (S. sanguis), Streptococcus salivairus (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 *Candida albicans* is aided by hyphal development. The transformation of budding *candida albicans* to hyphal growth is endorsed by physical contact with surfaces and is under genetic control. When *candida albicans* colonize an epithelial or epidermal surface, they adhere to host cells and create depressions in the surface of host cells. As *candida albicans* -form cells alter to the hyphal form, these hyphae are capable to diffuse into the surface of the tissue layer. The direction of hyphal growth is determined by the topography of the substratum. Hyphae are guided by ridges in the tissue layer. This behavior is known 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). Our result 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 [6,5,17,21] in which a high significant risk of mouth colonization was associated with bad mouth hygiene.

CONCLUSIONS

Treatment with a FOA may alter the ecology in the oral cavity by introducing new stagnant areas available for colonization and retention of *Candida albicans* and other *Candida* species. The results confirm this by indicating that FOAs have a direct effect upon the prevalence and density of *Candidal* carriage in this group of adolescents and young adults. The appliances may also interfere with oral hygiene practice as they cover considerable parts of the tooth surfaces with metal and composite materials. In clinical terms, these findings indicate that regular advice and routine instruction in oral and appliance hygiene given to this group of patients did not overcome completely the possible detrimental effects of OCAC. But rregular smoking and chewing Qat direct effect the OCAC in FOAs patients, Hence, particular attention has to be paid to the OCAC control of patients undergoing FOA therapy, also smoking and Qhat chewing 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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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	

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 candida albicans	29	13.8
Total Candida species	36	17.14

Table 3: Distribution of *Candida albicans* in relation to gender and age among Fixed Orthodontic patients

Characters	Positive *Candida 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 *Candida 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