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Prevalence of Candida Species in Patients with Fixed Orthodontic Appliance

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Abstract:

Background: Patients with tooth misalignment are typically treated with traditional brackets during orthodontic treatment. The complexity of bracket design often hinders proper oral hygiene, resulting in an increased load of microbiota. The shape of the brackets acts as a reservoir for *Candida*, leading to candidosis of the oral cavity.

Aims: This investigation aims to evaluate the impact of fixed orthodontic appliances on the count and distribution of salivary *Candida* species compared to saliva from subjects without orthodontic appliances. **Methods**: Eightyparticipants from the Iraqi population wearing fixed orthodontic devices comprised the study group, while 20 participants without orthodontic devices served as the control group. Salivary samples were obtained from both groups, and Candida chromogenic agar was used for cultivation. The counting and identification of different Candida species were confirmed using the Vitek 2 system to validate cultural characteristics.

Results: In this study, four types of Candida species were identified: *C.parapsilosis, C.albicans, C. tropicalis* & *C.krusei* in comparison to various published researchs, this study showed no statistically significant increase in colonization by any *Candida* species colonization during the orthodontic treatment. However, *C. tropicalis* was the most frequently isolated species for both test and control groups in comparison with other species. The fixed appliances had no impact on the existence, non-attendance or scale of habitation by *Candida* species and there were no considerable variation in the prevalence of *Candida* species, based on different factors (age, gender, and saliva pH) for both test and control groups.

Conclusions: The results of this study indicate that fixed orthodontic treatment had no effect on the prevalence of Candida species. This suggests that Candida accumulation may be related to an individual's own oral flora and is influenced by oral hygiene practices throughout their life.

Key words: *Candida albicans, C. tropicalis, C. glabrata, C. parapsilosis, C.krusei,* fix orthodontic appliance.

Introduction:

The third most common problem in oral health is malocclusion, which is responsible for many oral complications (1). Therefor, the aim of comprehensive orthodontic management is to improve patients' aesthetics, occlusion, mastication, self-respect and overall health (2). Classical brackets are commonly used in the treatment of patients with malocclusions. Oral hygiene can be affected by complex construction of brackets, which considers as predisposing factors to raised load of yeasts and bacteria (3, 4). In classical orthodontic brackets, one of the elements which are used to connect orthodontic wires with brackets is elastomeric rings. These rings are favorable conducive to biofilm formation and colonization of microorganisms due to their roughness and irregularity (5). The accumulation of various microorganisms leads to the formation of biofilms. It is style of life for many microorganisms to survive in hostile circumference, so it became as a reservoir of pathogens, biofilm consider as one of virulence elements of *Candida* and it would appear that isolated strains from orthodontic appliance will form a well biofilm (6).

In human oral cavity the common micro flora which colonizes up to 60% in young adults are *Candida* species (7), it may cause oral mycosis. *Candida albicans. C. tropicalis, C. glabrata, C. parapsilosis, C.krusei* are the most causative agents of oral candidosis especially in immune compromised patients such as diabetic, the presence of oral gram $_{+}$ ve bacteria, increase

the ability of Candida species to form biofilm (8).

The objective of this project is to evaluate the effect of fixed orthodontic appliance on the prevalence of salivary *Candida* species and compare with saliva of control group.

Materials and Methods:

The research was conducted in accordance with the guidelines of the Declaration of Helsinki, and was approved by the Ethics Committee of College of Dentistry, University of Mosul, Iraq (UoM. Dent/H.L. 18/22). Samples were collected from both Al-Noor and Al-Dhabat centers after obtaining approval from the Nineveh Health Department.

Eighty subjects (40 female and 40 male) age between 11 and 40 years, with a mean age of 19.6 years, wore fixed upper and lower orthodontic appliances for varying treatment intervals. They were compared to control group of 20 subjects. A single examiner assessed all patients, recording their name, gender, age, and responses to a questionnaire that focused on their oral hygiene habits, including the frequency of tooth brushing, use of interdental floss and mouthwash usage. Subjects in this study did not record any systemic disease, did not taking any antimicrobial drugs at the past three months, weren't smokers, there were no history for trauma, weren't wearing orthodontic appliance previously and facultative take part in the research. Subjects were ordered to not drink or eat at least two hours before collection of saliva. An stimulated saliva about 3ml was collected in sterile plastic tube with lids, then thermal receptacle with ice was used to transported saliva and processed within two hours after gathering, and the pH for each saliva was measured by chemical indicator paper strip (Ruttapharm madaus group). (9)

After shaking of saliva sample with shaker, Swab was used for streaking 100 µl saliva sample on HiChrome ™ Candida agar plates which prepared according to instructions of manufacture (Himedia) then incubated at 37C´ for 24-48 hours. After cultivation the number of *Candida* colonies on each plate were manually counted. Different Candida species were identified by colony culture characteristics particularly the color, and confirmed by using the VITEK 2 system automated card system. (10)

Statistical Analysis

The outcomes of the investigation were subjected to statistical analysis using SPSS version 26. To test the impact of orthodontic treatment on the prevalence of Candida spp as well as the effect of three parameters (age, gender, and saliva pH) in orthodontic patients on the prevalence of Candida spp, compared with control

individuals. Nonparametric Pearson's chi^2 test was utilized. Results with a significance level of *p* were less than 0.05 ($p < 0.05$) were considered statistically significant.

Results

The total number of samples in this study was 100, consisting of 80 in the test group and 20 in the control group. The test group was distributed by age, with 40 test and 10 control participants aged 20 or younger, and 40 test and 10 control participants over 20. By gender, there were 40 test and 11 control males, and 40 test and 9 control females. Regarding pH levels, there were 39 test and 11 control participants with a pH of 7 or less, and 41 test and 9 control participants with a pH greater than 7, as shown in the table (1).

Factor		Test	Control	Total
Age	≤20	40	10	50
	>20	40	10	50
Gender	male	40	11	51
	female	40	9	49
PH	≤7	39	11	50
	>7	41	9	50

Table(1): Samples distribution

100 µl of each saliva sample was aseptically cultured on Candida chromogenic agar plates which appeared in various color and colony characteristics. There was no statistical variation in the predominance of all types of Candida spp. Between the test group (patients wearing fixed orthodontic appliance) and control groups for both age groups (≤20 , >20) $p_=$(0.23, 0.09) respectively, gender male and female groups $p_=$(0.79,0.1) respectively, pH groups (≤7 &>7) $p_=$(0.35, 0.06) respectively. Table (2) showed the prevalence of the total number of Candida spp. For test and control groups.

For the cultivation and identification of Candida spp, swabs were taken from the oral cavity, allowing for an objective evaluation of the existence or exclusion of fungal habitation in the study groups of patients. In our study, four different Candida spp. Observed on chromogenic agar, the initial identification based on colony characteristics particularly the colors. The

ultimate identification were carried out by Vitek 2 system: *C. tropicalis, C.parapsilosis* : white, *C.albicans*: turquoise: blue & *C.krusei*: pale violet (Fig 1 a and b).

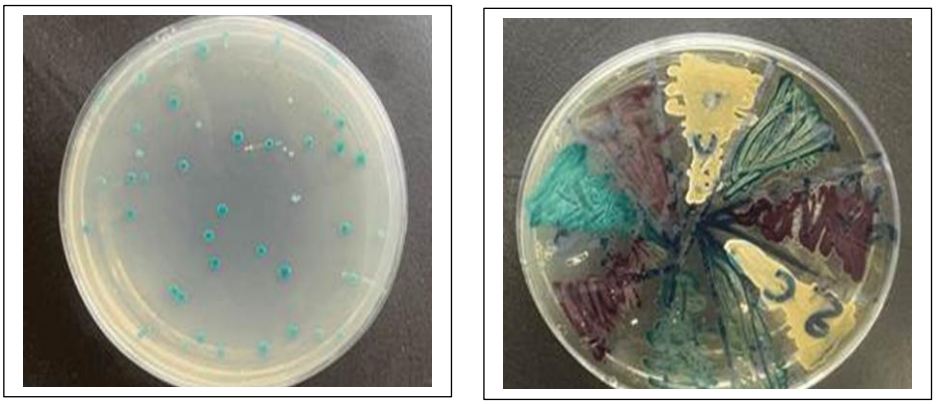

Fig 1: a *C.albicans* turquoise colony color, b different candida species isolates

The total number of all Candida species were counted for both test (orthodontic) and control individuals Table (2).

Factor		Test			Control			Sig.
		Absent	≤20	>20	absent	≤20	>20	
Age	≤20	17	18	5	7	3	0	0.23
	>20	17	12	11	1	6	2	0.09
Gender	Male	14	16	10	5	4	2	0.79
	female	20	10	10	4	5	0	0.1
PH	≤7	21	17	11	5	4	2	0.35
	>7	21	9	11	4	5	0	0.06

Table(2): The prevalence of candida spp in test and control groups

In contrast to other published studies, no statistically significant increase in Candida species colonization was observed after orthodontic treatment. The study, revealed the frequent presence of Candida spp. In each test group across the three parameters. In the age group ≤20 25 samples, representing 50% were free of all Candida species, while *C. tropicalis* appeared in 15 samples, representing 30%, *C. albicans, C. parapsilosis* & *C. krusie* were (1, 2, 1) representing (2%, 4%, 2%) respectively, for age group >20 Candida spp. Absent in 18 samples, representing 36.8% while again *C.tropicalis* were the most frequently appeared in 22 samples, representing 44.9%, other Candida spp. Were *C. parapsilosis* 3 (6.1%), *C. krusie* 1 (2%), *C.albicans* & *C.tropicalis* appeared together in 1 sample (2%), *C. parapsilosis* & *C. krusie* 1(2%), *C .krusie* and *tropicalis* 3 (6.1%) Table (3).

≤20				**>20**		
Spp.	**Freq.**	**%**		**Spp.**	**Freq.**	**%**
Non	25	50		Non	18	36.7
C.albicans	1	2		*C.parpsilosis*	3	6.1
C.parpsilosis	2	4		*C.krusi*	1	2
C.Krusi	1	2		*C.tropicalis*	22	44.9
C.Tropicalis	15	30		*C.albicans &C.tropicalis*	1	2
				C.Parpsilosis& C.krusi	1	2

Spp.: species, Ferq: frequency, %:percentage

Table (3): frequency of C. spp. For orthodontic individuals according to age groups

For the salivary pH group ≤7: 17 samples representing 34% were free of Candida spp. while *C. tropicalis* appeared in 19 samples representing 38%, *C. albicans, C. parapsilosis , C. krusie, Parpsilosis& krusi* together, *Parpsilosis& tropicalis together, krusie* and *tropicalis* together were (1, 4, 1, 1, 1, 3) representing (2%, 8%, 2%, 2%, 2%, 6%) respectively ,for pH group >7 Candida spp. Included absent in 25 sample representing 50 % while again *C.tropicalis* were the most frequently appeared in 19 samples representing 38 %, other Candida spp. *C. parapsilosis, C. krusie , parapsilosis & tropicalis* together, *C. krusie & tropicalis* together (3, 1, 1, 1) representing (6, 2, 2, 2 %) Table (4).

≤7				>7		
Spp.	Freq.	%		Spp.	Freq.	%
Non	17	34		Non	25	50
C. albicans	1	2		*C.parpsilosis*	3	6
C.parpsilosis	4	8		*C.krusi*	1	2
C.krusi	1	2		*C. tropicalis*	19	38
C.tropicalis	19	38				
C.parpsilosis& C. *krusi*	1	2		*C.parpsilosis*& *C. tropicalis*	1	2
C.parpsilosis& C.*propicalis*	1	2		*C.krusi*&C.*tropicalis*	1	2
C.krusi&C.*tropicalis*	3	6				

Spp.: species, Ferq: frequency, %:percentage

Table(4): frequency of C. spp. For orthodontic individuals according to Salivary pH groups

For gender male:19 samples representing 37.3 % were free of Candida spp. While *C. tropicalis* appeared in 22 samples representing 43.1%, *C. parapsilosis*, *C. Parpsilosis* & *C. tropicalis* together, *C. krusie* and tropicalis together were (3, 2, 5) representing (5.9%, 3.9 %, 9.8%) respectively, for female group Candida spp. Absent in 24 sample representing 49 % while again *C.tropicalis* were the most frequently appeared in 15 samples representing 30.6 %, other Candida spp. *C. albicans*,*C. parapsilosis*, *C. krusie* , *C.parapsilosis* and *C.tropicalis* together (1, 4, 1, 4) representing (2,% 8.2%, 2%, 8.2 %) Table (5).

Male				**Female**		
Spp.	**Freq.**	**%**		**Spp.**	**Freq.**	**%**
Non	19	37.3		None	24	49
C.Parpsilosis	3	5.9		*C.albicans*	1	2
C.tropicalis	22	43.1		*C.Parpsilosis*	4	8.2
C.parpsilosis& *C.tropicalis*	2	3.9		*C.krusi*	1	2
C.krusi& *C.tropicalis*	5	9.8		*C.tropicalis*	15	30.6
				C.parpsilosis&*C.tropicalis*	4	8.2

Spp.: species, Ferq: frequency, %:percentage

Table(5): frequency of C. spp. For orthodontic individuals according to gender groups

Discussion:

The oral cavity hosts various microorganisms, including bacteria, viruses, and fungi (11, 12). Some fungi are part of natural microflora and typically do not harm the host, suggesting a mutualistic relationship. However, some biologists view isolated fungal colonies as a form of parasitism, indicating potential harm (13). The presence of orthodontics fixed device can increase plaque accumulation, affecting the balance of bacteria and fungi in the mouth(14).

The research compared salivary Candida colonization between patients with fixed devices on permanent teeth (test group) and subjects without orthodontic appliances (control group). Samples were divided into test and control groups based on age, with two categories: subjects aged 20 or younger (40 test, 10 control) and those older than 20 (40 test, 10 control). The groups were also categorized by gender, with 40 test and 11 control males, and, 40 test and, 9 control females. Additionally, samples were classified according to pH with 39 test, 11 control ≤ 7& 41 test, 9control >7. Exclusion criteria were smokers, patients taking medications like immuno suppressant and antibiotics and those who had received additional orthodontic remedy also were omitted as any of these considerations were shown to induce changes in oral clonal expansion by Candida (15).

Different methods have been fortunately utilized for the separation and identification of Candida in oral, which include swab culture (16) and saliva culture (17)

There was no statistical difference in the prevalence of all types of Candida spp. Between the test (for patients wearing fixed orthodontic appliance) and control groups in both age groups (≤ 20,>20) p=(0.23, 0.09) respectively, gender male &female groups p=(0.79, 0.1) respectively, pH groups (≤ 7 and >7) p=(0.35, 0.06) respectively, according to our own perceptions, there was increase in the colonies number of Candida in the test group in comprising with control but this increasing was not significant and this outcome agree with Addy et al. showed no significant differences between sets of healthy adolescents without fixed brackets and remedy with fixed brackets. The researchers of the study proposed fixed orthodontic device may motivate candida reproduction in those who are candida bearer (18), likewise Dar-Odeh et al. (19) in a study manifested that fixed metallic orthodontic device did not promote oral Candida colonization through the first four months research time, other researchers Hagg et al. also founded an increase Candida colonization with fixed orthodontic device (20).

Candida species in patients wearing fixed orthodontic devices compared to those not wearing orthodontic appliances. Our results revealed that both groups showed an increased susceptibility to colonization *C.albicans* Candida tropicalis, rather than *Candida Tropicalis.* This finding contradicts what Hägg et al. reported, the predominant candida species isolated post wearing fixed orthodontic devices *C. albicans* (20), and also Hibino et al. wrought that *C. albicans* is an opportunistic microbe typically found the patients with orthodontic devices (21).

Conclusion:

Remedy with fixed orthodontic devices may alter the oral cavity's microbiota by creating new retentive areas, which can have a transitory impact on the count and prevalence of Candida species. However, our study found no significant variation between the test groups. This indicates that Candida is a part of the endogenous microflora and are considered commensal microorganism and it is portion of oral microflora. Additionally, the good oral hygiene practices of orthodontic patients by multiple brushing and mouthwash use, also influence the prevalence and count of Candida.

Conflict of interest

The researchers announced that they had no conflict of interest.

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