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Research Article

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The Effect of Denturewearing and Fixed Orthodontic Appliance on Increasing the Colonization Rate of Candida in the Oral Cavity

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Abstract

Background and Objectives: *Despite the fact that Candida spp. belong to the normal oral microbiota, living on the tongue, gums, palate and saliva of healthy individuals as commensal yeasts, they can cause oral or oropharyngeal infections. The objective of this study was to assess the colony forming unit (CFU) of oral candida from the buccal mucosa between 3 cluster of individuals who had removable or fixed prostheses (denture) and fixed orthodontic appliance (FOA) comparing with normal teeth control group.*

Subjects and Methods: *The investigational group was selected from denture and orthodontic patients whom were examined clinically as soon as to get baseline information before any active treatment for dental infections. The cluster included 104 denture patients; 104 fixed orthodontic appliance (FOA) patients and 102 controls. The study included 130 males, 180 females (mean age 37.01 ± 20.9 years). Clinical, demographic data and risk factors were collected in standard questionnaire then mucosa of each participants was first swabbed and then placed in liquid medium, and then, after successive dilutions were created and applied to the Sabouraud's dextrose agar, the CFU was calculated after 24 hours, or at what point a single layer of candida had formed on the Sabouraud's dextrose agar at any dilution level.*

Results: *There was a significant correlation between the increase of candida colonization of the bucall mucusa with the prosthesis (denture), and FOA where the mean ± SD was 96.2 ± 55.4 CFU/mL and 83.8 ± 51.7 CFU/mL respectively greater than 57.8 ± 45.2 CFU/mL for the normal controls, indicating the enhancement of the effect of the prosthesis and FOA on the heavy colonization of candida in the oral cavity ($p < 0.0001$). Additionally, there was no significant variation in the mean ± SD or difference in buccal CFU for oral Candida species in relation to sex and age groups.*

Conclusion: *Patients with dentures and FOA showed higher buccal CFU readings than normal controls without prostheses, indicating a higher risk of plaque adhesion in patients with dentures and FOA.*

Keywords: Buccal CFU, Denture, Fixed Orthodontic Appliance (FOA), Oral Candida Colonization (OCC), Yemen.

Introduction

A deeper comprehension of the various microbes that reside on or within human tissues and fluids has been made possible by developments in metagenomics. The term "normal microbiota" refers to the variety and quantity of these micro-organisms that are present in the human body and are influenced by factors such as age, food, hygiene practices, and prosthetic status. Specifically, the oral cavity is colonized by a wide variety of microorganisms from various species, such as fungi, viruses, bacteria, and protozoa [1, 2]. They are always found on the surface of oral mucosa, gums, teeth and tongues in highly ordered, metabolically interdependent polymicrobial communities. The remaining, less common and more diverse microbiota is known as the "rare biosphere," whereas the bacterial microbiome, which accounts for more than 99% of all microbial counts, is referred to as the core microbiome [3]. A large fraction of the rare biosphere is comprised of the mycobiome. 85 fungal genera were identified in healthy hosts in culture-independent investigations; the most common genera were Candida, Cladosporium, Aureobasidium, Saccharomycetales, Aspergillus, Fusarium, and Cryptococcus

[4]. Aspergillus, Cladosporium, and Penicillium spp. were the most common molds in culture-dependent investigations using salivary samples, whereas Rhodotorula and Candida spp. were the most prevalent yeasts [5]. According to these researches, the most common species found in the oral cavities of up to 70% of healthy people were Candida species [6, 7]. In actuality, Candida species such as Candida albicans, Candida dubliniensis, Candida parapsilosis, and Candida glabrata are part of the typical skin and mucosal surface microbiota of healthy persons, and they are found in the gastrointestinal tract, the vagina, and the oral cavity [8]. The role of the mycobiome in human health and disease is well established, despite its very low abundance [9]. A range of oral mucosal illnesses, from simple to chronic candidosis, are caused by Candida opportunistic infection, which is facilitated by weakened host defenses or insufficient clearance [10, 11]. Commensal fungi can multiply, enter the bloodstream, and spread throughout the human body in severely immunocompromised patients, such as those receiving chemotherapy, AIDS patients, patients with endocrine or blood diseases, or patients receiving treatment for other conditions, posing a serious risk of infection [8, 12-14].

Most studies on the prevalence of C. albicans in the human oral cavity have been performed in individuals with different pathologies or belonging to risk groups. It is widely described that C. albicans predominates among Candida spp. in the oral cavity, but little is known about stability and transmissibility between individuals. Hence, the objective of this study was to assess the colony forming unit (CFU) of oral candida from the buccal mucosa between 3 clusters of individuals who had removable or fixed prostheses (denture) and fixed orthodontic appliance (FOA) comparing with normal teeth control group.

Materials and Methods

Candida tests were performed on 310 individuals (104 with dentures, 104 with orthodontic abaratus, and 102 controls without dental prostheses) over the course of 2 year, starting in Jounuary 2022 and ending in Junuary 2024, in the dental clinics of the Faculty of Dentistry, Sana'a University, Yemen, and departement of Medical microbiology laboratory, Faculty of Medicine and Health Sciences. Inclusion criteria for subject selection were healthy individuals with no clinical signs of Candida infection no systemic disease. In addition, individuals who smoked, currently taking antifungal, steroids, antibiotics, or immunosuppressive drugs in the past 6 months were excluded.

Sample Size and Power

Using computation software, the sample size was established by comparing the rates of variance between controls and cases, or patients with dental prostheses. We require 102 participants in each group if the ratio of change in CFU counts for the control group is 2% and for the cases is 20%, with a 99.9% confidence level and power equal to 80%. A total of 310 subjects—104 wearing dentures, 104 undergoing orthodontic treatment, and 102 serving as controls without dental prostheses—were chosen; the remaining five cases did not result in any problem.

Collection of Patient Sample for Candida Count (CFU)

Each patient and control had two sterile cotton swabs drawn from them to collect samples. Swab samples from three groups were placed in Stuart transport medium before being delivered to the microbiology laboratory. At two separate points in the mouth of the patient, buccal mucosa oral swabs were taken [15-17]. Before culture, the collected swab was dissolved in 1 ml of phosphate-buffered saline and stored at -20°C. The sample was then used for CFU calculation, culture and Candida species determination.

Candida Dilution

Candida generally grow at different densities, although maximum densities vary greatly depending on the species of Candida and the substrate in which they are grown. To produce easily countable numbers of Candida, a series of dilutions must be made and each one must be titrated by one or two dilutions. Ten-fold serial dilutions of Candida covering all were prepared. Next, 0.1 ml of each dilution was transferred and placed on top of prepared Sabouraudus dextrose agar. Two samples were cultured in duplicate using Sabouraudus dextrose agar. Next, the culture medium was incubated for 24 to 48 h at 37°C.

Colony Forming Unit (CFU)

Only plates containing between 30 and 300 colonies were counted (or repeated plates from the same dilution); plates containing more than 300 colonies were rejected.

Culturing Candida

On Sabouraudus dextrose agar, 100 µl of the concentrated oral rinse was added, and the mixture was incubated for 48 hours at 370C. At -20oC, the long-term samples were kept. In case Candida colonies emerged on Sabouraud dextrose agar, 100 µl of the swab oral rinse supernatant was used to inoculate chromogenic Candida agar, which was subsequently incubated for 48 hours to examine the colonies. Using the manufacturer's color reference guide, the colonies' colors were used to identify the species of Candida. A fermentation assay involving sucrose, maltose, glucose, lactose, and galactose was conducted when color identification was ambiguous. The capacity of Candida species to generate chlamydo-spores on glutinous rice agar was another method used to identify them [18].

Statistical Analysis

Epi-info Statistics version 7 was utilized to examine the information. The average and standard deviation (SD) of each graph and all data were presented as mean standard error of the mean (SEM) in the table. The Shapiro-Wilk normality test was used to determine and confirm that the data had a normal distribution ($p>0.05$). The Levene test findings were examined to determine homogeneity or uniformity of variance (homogeneous if $p > 0.05$). An independent-T test was used to compare the means of CFU oral candida from the buccal mucosa of the control and cases groups. The data gathered was normally distributed. A colony-forming unit (CFU) is a unit used to describe how many colonogenic cells are viable in a milliliter of solution extracted from the cotton swab. These provide a rough estimate of the number of cells that are still viable, capable of dividing, and forming small colonies. CFU/ml is determined by multiplying the total number of colonies by the dilution factor and dividing the result by the size of the culture plate. CFU/ml = (Colony Count * Dilution Factor) / Culture Plate Volume [15].

Ethical Consideration

On Jounuary 1, 2022, the Medical Ethics and Research Committee of Sana'a University's Faculty of Dntistry granted ethical per-

mission for Contract No. 317 project. The ethical principles set forth by the review committee were consistently followed. The chosen participants provided their informed, signed consent.

Results

The study included 310 individuals, 104 with dentures, 104 with orthodontic abaratus, and 102 controls without dental prostheses, 41.9% males and 58.1 females, ranging in age from 9 to 90 years, with a mean ± SD of age equal to 37.01 ± 20.9 years old. Most of the participants were in the age group 21–30 years (25.8%), followed by ≥51 years (23.9%) and 31–40 years (22.3%). The rate of candida colonization was 34.8% (108/310) (Table 1).

Table 1: General characteristics of participate in the study

Characters	N (%)
Sex	
Male	130 (41.9)
Female	180 (58.1)
Ages (years)	
<21 years	50 (16.1)
21-30	80 (25.8)
31-40	69 (22.3)
41-50	40 (12.9)
≥51	74 (23.9)
Mean age	37.01Years
SD	20.9 Years
Mode	23 Years
Median	26 Years
Min-Max	9- 90 Years
Type of patients	
Denture	104 (33.5)
orthodentic	104 (33.5)
Normal	102 (32.9)
Total	310 (100)
Positive colonization	108 (34.8%)

Table 2 shows the oral Candida colonization rate (CFU/ml) in the buccal mucosa of denture and orthodontic patient cases compared with normal controls. For denture patients, the mean ± SD of the buccal Candida count was 83.8 ± 51.7 CFU/mL, with mode equal to 37 CFU/mL, the median was 60 CFU/mL, and ranged from 22 to 171 CFU/mL, with the interquartile range being 75% (IQR) equal to 122 CFU/mL. The variance in all individual values was significantly distributed over the normal curve with a 6.9 t-test and $p < 0.001$. For denture patients, the mean ± SD of the buccal Candida count was 96.2 ± 55.4 CFU/mL, with mode equal to 23 CFU/mL, the median was 88 CFU/mL, and ranged from 23 to 211 CFU/mL, with the interquartile range being 75% (IQR) equal to 115 CFU/mL. The variance in all individual values was significantly distributed over the normal curve with a 6.3 t-test and $p < 0.001$. For non-prosthesis controls, the values were significantly lower than those of the denture or orthodontic patients; the mean ± SD of the buccal Candida count was 57.8 ± 45.2 CFU/mL, with mode equal to 15 CFU/mL, the median was 44 CFU/mL, and ranged from 11 to 160 CFU/mL, with the interquartile range being 75% (IQR) equal to 95 CFU/mL. The variance in all individual values was significantly distributed over the normal curve with a 6.1 t-test and $p < 0.001$ (Table 2). Table 3 shows the mean ± SD and difference in buccal CFU for oral Candida species in relation to sex, age, and the presence of dentures or orthodontic appliances. There was a significant correlation between the increase of candida colonization of the buccal mucosa and the denture wearer patients, where the mean ± SD was 83.8 ± 51.7 CFU/ml greater than 57.8 ± 45.2 CFU/ml for the normal controls, indicating the enhancement of the effect of the denture wearing on the heavy colonization of Candida in the oral cavity among the denture patient group ($p = 0.007$) with a significant difference of 26 CFU/ml between the 2 groups ($p = 0.007$). Also, there was a significant correlation between the increase of candida colonization of the buccal mucosa and the orthodontic appliance patients, where the mean ± SD was 96.2 ± 55.4 CFU/ml greater than 57.8 ± 45.2 CFU/ml for the normal controls, indicating the enhancement of the effect of the orthodontic appliance wearing on the heavy colonization of Candida in the oral cavity among the orthodontic appliance patient group ($p = 0.0002$) with a significant difference of 38.4 CFU/ml between the 2 groups ($p = 0.0002$). There was no significant variation in the mean ± SD or difference in buccal CFU for oral Candida species in relation to sex and age groups (Table 3).

Table 2: Oral Candida colonization rate (CFU/ml) in the buccal mucosa of denture and orthodontic patient cases compared with normal controls.

Characters	Denture patients CFU/ml	Orthodenteric patients CFU/ml	Normal controls CFU/ml
	Buccal counts	Buccal counts	Buccal counts
Mean	83.8	96.2	57.8
SD	51.7	55.4	45.2
SE	12.2	15.3	9.4
Min	22	23	11
Max	171	211	160
Mode	37	23	15
Median	60	88	44
25% ile	41	56	21
75% ile	122	115	95
T-test	6.9	6.3	6.1
Df	17	12	22
P-value	<0.0001	<0.0001	<0.0001

Table 3: Mean ± SD and difference in buccal CFU for oral Candida species in relation to sex, age, and presence of dentures or orthodontic appliance.

Characters	N	Mean ± SD CFU/ml	Difference	SE	95% CI	t-test	DF	p
Male	130	76.3 ± 46.5	-1	8.4	-17.6-15.6	-0.1	153	0.90
Female	180	75.3 ± 55.1						
Ages								
<21 years	50	85.4±62.3	Reference					
21-30	50	91.3±66.9	5.9	16.6	27.3-39.1	0.35	63	0.72
31-40	80	67.2±47.8	-18.2	14.3	-46.9-10.4	-1.2	57	0.20
41-50	69	68.6±49.1	-11.8	17.3	-46.8-23.1	-0.68	42	0.49
≥51	40	87.7± 50.6	7.3	14.4	-21.5-36	0.5	60	0.61
Patient groups								
Normal	104	57.8 ±45.2	Reference					
Denture	104	83.8 ± 51.7	26	9.5	7.1-44.9	2.7	102	0.007
orthodontic appliance	102	96.2 ± 55.4	38.4	9.95	18.6-58.1	3.9	101	0.0002

This procedure calculates the difference between the observed means in two independent samples. A significance value (P-value) and 95% Confidence Interval (CI) of the difference is reported. The P-value is the probability of obtaining the observed difference between the samples if the null hypothesis were true. The null hypothesis is the hypothesis that the difference is 0

Discussion

There is ample evidence about the effects of yeast colonization on human health and illness [9]. This study assessed oral yeast colonization in healthy individuals, FOA, and denture wearers using culture-dependent identification techniques. Yeasts colonized thirty-four percent of the volunteers, which is within the wide range of reports in the literature (18.5% to 92.5%) [5, 19]. This wide range could be caused by a variety of potential confounding factors, such as the presence of dental prosthesis, age, ethnicity, and neglected co-infection. Microbiological culture variables could also be the cause, such as the type of sample collected, culture media, incubation time, or temperature [20]. Regarding oral Candida species, there was no discernible difference in buccal CFU or mean ± SD among sex groups in the current investigation (Table 3). Our findings are consistent with those of previous research, which found that neither the proportion of yeast carriage nor the CFU counts were impacted by a person's gender or oral hygiene practices (frequency of cleaning teeth) [5,19]. However, Burcham et al.'s study [21], which involved a crowdsourced population study, found that factors such as age, gender, and oral health practices like flossing frequency have an impact on young people's oral microbiomes. Also, the current result was similar to that reported by Thein et al. in which similar colonization rate was observed in both adult sexes [22].

Regarding age groups, there was no discernible difference in buccal CFU or mean ± SD for oral Candida species in the current investigation (Table 3). Our findings differ from those of Al-Ha-

dad et al., who observed a pattern of oral candida colonization growing with age in both FOA patient cases and healthy control subjects, with the older age groups showing the highest rate of candida colonization [23]. The results of the current investigation also differed with those of Chopde et al., who found that oral candida colonization is more common in older age groups [24]. The elderly's propensity for systemic disorders, dietary alterations, and altered salivary features can all be used to explain this conclusion [24, 25]. Growing older was also associated with an increased incidence of denture stomatitis in the elderly due to a decrease in cell-mediated immunity, which guards against Candidal infection. Some oral environmental parameters, like the age of the individuals and the unstimulated salivary flow rate, were linked to increased numbers of microorganisms in the saliva of complete denture wearers, according to Dar-odeh Shehabi et al. [26]. According to those authors, a rise in the concentration of microorganisms in saliva was caused by an aging-related decrease in salivary flow rate [27].

The study found a noteworthy relationship between increased Candida colonization in patients wearing dentures of the buccal mucosa and a mean ± SD of 83.8 ± 51.7 CFU/ml, which was higher than 57.8 ± 45.2 CFU/ml for normal controls. This suggests that wearing dentures promotes the occurrence of heavy Candida colonization in the oral cavity ($p = 0.007$). The ability of Candida species to form biofilms is one of its most important virulence factors. This ability has important clinical consequences as it confers resistance to antifungal therapy capacity for yeast cells within the biofilms to resist host immune defenses, which explains the highly significant association between denture wear and an oral Candida contract [21, 22, 28, 29]. A secondary explanation could be that oral microbiota can alter as a result of denture wear or tooth loss, which alters the oral environment [16].

Also, there was a significant correlation between the increase of candida colonization of the buccal mucosa and the orthodontic appliance patients, where the mean ± SD was 96.2 ± 55.4 CFU/ml greater than 57.8 ± 45.2 CFU/ml for the normal controls, indicating the enhancement of the effect of the orthodontic appliance wearing on the heavy colonization of Candida in the oral cavity among the orthodontic appliance patient group ($p = 0.0002$).This result is similar to the Shoga Al-Deen et al. study that explored a high oral candida colonization rate through fixed orthodontic therapy and 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 [30, 31]. The OCC 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 of this observation, as they interfere with thorough brushing of the gingival area [31]. Similar changes in OCAC rate during orthodontic treatment with removable and fixed appliances have also been reported by several authors [32-35]. Furthermore, the presence of rough-surfaced bonding material in FOA or dentures acting as a Candida trap and causing gingival irritation may have played a causative role [23, 34, 36-40]. Thus, a significant increase in oral candida colonization after the introduction of FOA 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 [31]. Also, it may be assumed that foreign substances, including appliances or dental prostheses, change the oral natural environment by mechanisms at present unidentified, such as the propagation of microorganisms. On the other hand, a number of researchers have revealed that the existence of a prosthesis or an appliance enhances candidal numbers [38-43].

Conclusion

The significant difference in bacterial load between prosthesis/FOA patients and non-prosthesis patients suggests that the presence of these abaratuses in the oral cavity may interfere with or deteriorate oral health. However, this effect is different from that of natural teeth because prostheses and FOA do not directly affect the surrounding oral flora like actual teeth do. Additionally, patients with prosthesis/FOA had greater buccal CFU readings than non-prosthesis/FOA patients, suggesting that prosthesis/FOA are more prone to plaque adhesion.

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A Dispute of Interest

Regarding this project, there is no conflict of interest.

Author's Contributions

First author Ebtihal Mohamed Madar did the fieldwork for this study as part of a PhD in the department of Medical Microbiology, Faculty of Medicine and Health Sciences, Sana'a university. Additional authors assisted with data analysis, drafting and reviewing the manuscript, and giving final clearance to the study.

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