

*Full Length Research Paper*

## **Fungal contamination of dental appliances: A cross-sectional study**

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Orthodontic treatment makes necessary to use a longtime fixed-band appliance, it offers suitable conditions for fungal growth, dentures also allow fungal contamination on their surfaces. The aim of our study is the identification of the fungal species colonizing surfaces of dental appliances, the cross-sectional study was carried out on 60 patients wearing dental appliances, during 5 months, and sterile swabs were used and inoculated into Sabouraud's dextrose agar tubes. Yeast identification has been based on germ tube test, chlamydoconidia production and biochemical tests (Auxacolor, Api 20 C). The overall prevalence of fungal contamination of dental orthodontic appliance was 35%. The prevalence rate was 40% among patients who had dental appliances for 1 to 2 months, and 45,5% fort patients brushing their teeth at least twice daily, but no statistical relation with mycosis was found. Pain, burning and discomfort were correlated to fungal carriage  $p=0,038$ ;  $OR=4,867$  IC 95% (1.09-21.74). The fungal species most recovered were *Candida parapsilosis* 21.47%, *Candida Zeylanoides* 21.47%, *Candida albicans* 17.38%, and *Cryptococcus terreus* 13.04%. Mycological monitoring of dental prosthesis is crucial to prevent possible fungal's adverse health effects.

**Key words:** Orthodontic appliances, dentures, fungal contamination, *Candida* sp, *Cryptococcus terreus*.

### **INTRODUCTION**

In recent years dental infections and biosecurity attracted greater interest of health professionals due to the spread of infectious diseases such as AIDS and Hepatitis B (Moreira et al., 2016). Oral appliances are divided in

prosthodontics and othodontics, ranging from braces and retainers to dental crowns, fillings and dentures.

Denture stomatitis, is a common inflammatory lesion, the erythema is localized to the fitting surface of the

denture bearing areas of maxillary removable dental prosthesis (Neville et al., 2009). At one time oral fungal infections were a relatively uncommon event, but with advances in health care and an increasingly aging population, oral fungal infections are becoming more common. Stomatitis appears to be multiparametric; old age and concomitant decline of the immune defences, systemic diseases, smoking, ill-fitting denture and poor oral hygiene resulting in the accumulation of plaque on the dentures have all been proposed as predisposing factors (Barbeau et al., 2003; Grimound et al., 2005; Figueiral et al., 2007). The use of oral appliances is usually asymptomatic; however, it may cause mild soreness or burning sensation.

Fixed orthodontic treatment can lead to alteration in the oral environment. A study revealed that orthodontic treatment is correlated to an increase in the count of microorganisms, an increased accumulation of plaque, and a decrease in the level of pH, which leads to a major risk of caries and periodontal diseases (Gujar et al., 2020). Orthodontic treatment leads to significant changes in both supragingival and subgingival microbial flora showed that there was an increase in the level of subgingival pathogens after the placement of orthodontic appliances (Gujar et al., 2020).

Numerous fungi are potentially pathogenic microorganisms, *Candida albicans* is the principal species associated with human infections ranging from the more common oral thrush to fatal systemic superinfections in patients who are afflicted with other diseases (Krishnan, 2012). Besides *Candida* sp, other fungi can cause oral diseases in humans: *Aspergillus*, *Cryptococcus*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Zygomycetes* class, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Penicillium marneffeii*, *Sporothrix schenckii*, and *Geotrichum candidum* (Neville et al., 2009; Samaranyake and MacFarlane, 1990). This study aimed identifying species's colonizing dental appliances surfaces.

## MATERIALS AND METHODS

This cross-sectional study was conducted among patients wearing dental appliances, 60 patients were submitted to a detailed clinical interview and oral clinical examination, and were instructed to keep their dentures. After obtaining informed consent and before collecting samples, a questionnaire was performed among dental wearers. From literature various factors may contribute to increase or decrease in fungal growth these may include oral hygiene of patient, immune status of patient and the medication patient is taking. So, all these factors were considered in this study. Sixty patients wearing dental appliances were selected according to following criteria: Patient included were clinically healthy; they had

good oral hygiene and clinically normal oral mucosa, and had no any kind of medication, the study received approval from the ethical committee.

After obtaining informed consent and before collecting samples, a questionnaire was performed among oral appliances wearers; it included (presence of carries, pain, or odour, teeth brushing per day, toothbrushing duration, prior treatment, type of appliance). Swabs were taken according to a standard protocol. Samples were taken of each appliance surface by means of sterile swabs (that is, each was left in place for 30 s), samples were obtained by swabbing a region (1 × 5 cm line) of the anterior flange of the appliance. Orthodontic appliances were assessed using sterile swabs on the metallic braces (Figure 1).

All samples were inoculated on Sabouraud dextrose agar (SDA) medium and incubated at 30°C for 7 to 14 days, to allow filamentous fungi and yeasts to grow. Identification of species was performed by phenotypical tests (germ tube formation, chlamidoconidia production), and with the aid of commercially available systems like biochemical carbohydrate fermentation and assimilation using Api 20c and Auxacolor. Data were managed and analyzed using statistical software 17.0 (SPSS, Inc., Chicago, IL).

## RESULTS

The 60 patients using dental appliance enrolled in this study had a mean age of  $48 \pm 2.62$  years, the age range of the participants was between 10 and 79. The 60 dental appliances were composed of 34 prosthodontics (dentures) and 26 orthodontics appliances (braces). The overall prevalence of fungal contamination of dental appliances was 35% (21/60). The specific prevalence of fungal contamination of denture was 38% (13/34), and the fungal contamination of braces was 31% (8/26). Dentures were the most contaminated appliances. Pain, burning and discomfort were correlated to fungal carriage  $p=0.038$ ;  $OR=4.867$  IC 95% (1.09-21.74) (Table 1).

Yeasts of *Candida* genus were present in 20 (86.96%) of positives appliances. *Candida parapsilosis* and *Candida zeylanoides* were the species most recovered in this study (21.74% respectively). The various species are outlined in Table 2. Two samples were polymicrobials with the concomitant presence of *C. parapsilosis* and *C. zeylanoides*.

## DISCUSSION

Denture-induced stomatitis is an inflammatory reaction of the denture-bearing mucosa that affects approximately 65% of complete upper denture wearers (Webb et al., 1998), in our study fungal contamination was more important among dentures.

Fungi and protozoa may find favorable conditions to thrive in dental units. Literature papers have reported

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**Figure 1.** Orthodontic appliance infected by *Candida albicans* strain.

**Table 1.** Individual risk factors and their correlation with appliances fungal carriage.

Variable	Fungal +	Fungal -	OR	IC 95%	P
Age	-	-	-	-	NS
Sex M/F	8/13	15/24	1.161	0.341-3.026	NS
Nbr brushing per day 1-2/more than 2 times	-	-	1.563	0.497-4.913	Ns
Caries +/-	16/5	31/8	0.826	0.232-2.940	NS
Pain +/-	18/3	25/14	4.867	1.09-21.74	0.038
Odour +/-	-	-	0.747	0.253-2.207	NS
Residence urban/rural	13/8	17/22	0.464	0.143-1.502	NS
Toothbrushing duration Less than 1 min/more than 1 min	-	-	0.800	0.251-2.545	NS
Prior treatment +/-	13/8	23/16	1.131	0.381-3.354	NS
Type of appliance	-	-	1.525	0.773-3.009	NS

**Table 2.** Fungal species recovered from dental appliances surfaces.

Species	N	Percentage
<i>Candida parapsilosis</i>	5 (3 dentures, 2 braces)	21.74
<i>Candida zeylanoides</i>	5 (2 dentures, 3 braces)	21.74
<i>Candida albicans</i>	4 (1 denture, 3 braces)	17.38
<i>Cryptococcus terreus</i>	3 (2 dentures, 1 braces)	13.04
<i>Candida kefyr</i>	1 (1 denture)	4.35
<i>Candida pelliculosa</i>	1 (1 denture)	4.35
<i>Candida rugosa</i>	1 (1 braces)	4.35
<i>Candida famata</i>	1 (1 denture)	4.35
<i>Candida boidini</i>	1 (1 denture)	4.35
<i>Candida glabrata</i>	1 (1 denture)	4.35
Total	23	100

microorganism counts ranging from 100 to 400.000 CFU/ml in dental units (Kim et al., 2000; Ozcan et al., 2003). It is usually asymptomatic; however, may cause mild soreness or burning sensation in some (Krishnan, 2012), we found a strong correlation between fungal presence and soreness or burning sensation ( $p=0.038$ );  $OR=4.867$  IC 95% (1.09-21.74). Poor hygiene of dentures is also associated with fungal stomatitis (Grimound et al., 2005; Budtz-Jørgensen, 1978). Disinfection of dentures and dental appliances plays a key role in eradicating this form of candida infections (Muzyka, 2005), but number of brushing per day and duration of brushing teethes was not linked to presence of fungi in our study.

In a previous study, the duration of denture use was ranging from 3 weeks to more than 40 years, was generally unrelated to degree of contamination (Glass et al., 2001), in concordance with our results. On the other hand, Gujar et al. (2020) found that the microbial contamination in metallic brackets was higher than that of aligners, when used for a month. The porosity of the acrylic allows fungal and bacterial contamination throughout the entire denture (Glass et al., 2001). Moreover, the relatively acidic and anaerobic microclimate underneath the denture provides an ideal environment for yeast growth, and the denture shields the mucosa from the saliva and its local immunity-enhancing properties. Thus, the denture or any acrylic-containing dental appliance can serve as a source of inoculation for bacteria and fungi (Muzyka, 2005).

*Candida* species may be recovered from up to one-third of the mouths of normal individuals and are considered inhabitants of the normal flora of oral and gastrointestinal tract (Krishnan, 2012). However, the involvement of *Candida* as the main causative agent in denture-induced stomatitis (Pinto et al., 2008) was first described by Cahn in 1936, *Candida albicans* remains the most frequently isolated fungal in the oral cavity but other species have also been isolated and involved in disease (Garg et al., 2012), *C. albicans* occurred in the third place. The most commonly occurring dental fungal infection is caused by *Candida* species with *C. albicans* most often encountered (Muzyka, 2005), this is in accordance with our results.

Filamentous fungi can also be considered as biofilm-forming organisms because they are well adapted to grow on both biotic and abiotic surfaces. The formation of biofilms (Damasceno et al., 2017), but we did not recorded any filamentous fungi in this study.

*In vitro* adherence studies illustrate that *C. albicans* attaches readily to various resins, glass, and metal surfaces. The ability of *C. albicans* to adhere to polymeric surfaces has been correlated with attractive hydrophobic and repulsive electrostatic forces (Garg et al., 2012).

In denture wearers, Candidiasis is aggravated by the adhesion of *C. albicans* to the tissue surface of the maxillary denture base, which serves as an effective

reservoir of microorganisms (Garg et al., 2012).

Of the organisms recovered in this study, *C. parapsilosis*, *C. famata*, *C. rugosa* and *C. glabrata* are most frequently involved in human infections. Species that were once considered to be saprophytic are becoming more commonly associated as opportunists causing human disease.

In another study, *Candida* species isolated from complete upper denture wearers were *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. guilliermondii*, *C. krusei*, *C. lusitanae*, *C. tropicalis* (Pinto et al., 2008), *Candida kefyr* and *Cryptococcus* were involved in oral fungal infections (Krishnan, 2012).

Basically, the present analysis has pointed to a high prevalence of fungal organisms in oral appliances, in another study samples obtained at both peri-implantitis (31.6%) and healthy (40%) implant sites were dominated by *Candida* sp (Schwarz et al., 2015), which is in accordance with our results.

*C. albicans*, *C. boidinii*, and *Rhodotorula* sp have already been identify by Polymerase chain reaction (PCR) in oral peri-implantis sites (Schwarz et al., 2015), *C. glabrata* was also found in denture appliance (Glass et al., 2001).

*C. boidinii* is widespread and it has been isolated from diverse substrate related to human activity (wine fermentation, olive manufacturing, tepache), and natural environments (soil, seawater, sap fluxes of many sugar rich tree species (Camiolo et al., 2017).

Cryptococcosis also infects the central nervous system, skin and the oral mucous membrane (Glick et al., 1987), it can produce a variety of cutaneous and oral mucosal manifestations such as superficial ulcers, nodules, granulomas or carcinoma-like lesions (Myrvik and Weiser, 1988). Intraoral sites commonly affected are gingiva, palate and tooth socket after extraction. Violaceous nodules of granulation tissue, swellings and ulcers are the various forms of oral lesions reported (Samaranayake and MacFarlane, 1990). The numerous opportunistic and pathogenic microorganisms found in this study were unexpected and are known to produce not only substantial oral infections, but also systemic diseases as mentioned in previous studies (Pinto et al., 2008). Furthermore, the present analysis failed to identify any significant correlation of either fungal organisms or disease severity with risk factors, such as caries and teeth brushing.

## Conclusion

*Candida* species are assumed to cause disease by direct tissue invasion, either by inducing a hypersensitive state or by producing potent *Candida* toxins. The use of oral appliances is known to increase the carriage of *Candida* in healthy patients, and the proliferation of *Candida* sp strains can be associated with denture-induced

stomatitis. Being aware of these fungal risks, disinfection of dental appliances must be reinforced, and it is desirable to institute procedures to minimize the number of potentially fungal pathogenic agents.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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