original research article

THE OCCURRENCE OF *CANDIDA* SPP COLONISATION AND DENTURE RELATED STOMATITIS IN PATIENTS WEARING NEW DENTURES

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SUMMARY
Objectives. The *Candida* related denture stomatitis is a condition characterized by inflammation of the mucosa covered by the denture. The aim of the study is to evaluate the occurrence of Candida colonization and the eventual denture stomatitis, in totally edentulous patients after six and twelve months since the replacement of their dental prosthesis.
Methods. Oral swabs were collected at time 0, time 1 and time 2 from palatal mucosa and tested for *Candida species*.
Results. Out of 138 oral swabs at time 0 C. albicans were isolated in 80,70%, C. glabrata 8,77 %, and co-colonisation C. albicans/C. glabrata 7,01%. At time 1, oral swabs resulted positive were 27,53% : C. albicans were isolated in 50%, C. glabrata 23,68% and co-colonization C. albicans/C. glabrata in 26,31%. Among patients with positive oral swabs, we found denture-stomatitis in 25 patients. At time 2, oral swabs resulted positive were 68,11%: C. albicans 52,12%, C. glabrata 26,59% and co-colonization of C. albicans/C. glabrata 21,27% . Mucosal clinical examination showed signs related to denture stomatitis in 83 patients with oral swabs positive for Candida spp.
Conclusion. C. albicans remains the main etiological agent accounting up to 80%, however C. glabrata has emerged as a pathogenic agent of the oral mucosa, either co-infecting with C. albicans. Our data highlight an association between *Candida* spp colonization and clinical features of DRS: patients who develop a C. albicans DRS tend to have a more severe compared to patients with C. glabrata DRS. The presence of a co-colonization with C. albicans and C. glabrata infection is associated with a more severe clinical form of DRS. The time factor also plays a role in respect of the mechanical-action carried out by the same prosthesis pressure towards the oral epithelium and the possible role of this factor in altering the local immune balance.

Key words: *Candida* spp, denture related stomatitis, mechanical stress.

Background

Candida albicans is a commensal fungus, but circumstantially it may cause superficial infections of the skin and mucous membranes, such as denture stomatitis.
This condition is characterized by inflammation, chronic erythema and edema of the palatal mucosa, particularly in areas in contact with the denture acrylic surface (1, 2).
Epidemiological studies has reported a prevalence of denture related stomatitis (DRS) among denture wearers ranging from 15 to over 70% (1-4).
Etiological factors include poor denture and oral hygiene, continual wearing of removable dentures, as well as microbiological contamination with biofilm formation on denture surface. In addition, poor-fitting dentures can increase mucosal trauma. All of these factors taken together

are able to increase the ability of *Candida albicans* to colonize both the denture and oral mucosal surfaces, where it acts as an opportunistic pathogen (3, 5, 6).
Approximately 90% of cases of DRS are triggered by yeasts, typically *Candida albicans*, although other species, such as *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida guilliermondii* and *Candida dubliniensis*, may also contribute to the pathogenesis of the disease (7, 8).
Antifungal treatment can eradicate *Candida albicans* contamination and relieve stomatitis symptoms, but unless dentures are decontaminated and their cleanliness maintained, stomatitis recurs when the antifungal therapy is discontinued. The management of *Candida albicans* denture-stomatitis includes denture cleaning and disinfection, appropriate denture wearing habits, refitting dentures by applying tissue conditioners or soft liners, and topical or systemic antifungal therapy (9-15).
Impregnating the dentures of antifungal drugs, that are then eluited in the oral cavity, although is a therapeutic strategy often used, has some limitations. The drug is eluited in a concentration higher than the minimum inhibition concentration in the proximity of the denture only and the antifungal activity lasts for some weeks, resulting not effective at long term (16-22).
The mechanical stress of the denture might have also a pivotal role in the insurance of denture stomatitis as well as its maintenance, on the epithelials cell and the balance between immune local response and oral microbiota.
So, the aim of the study is to evaluate the occurrence of *Candida* colonization and the eventual denture stomatitis, in totally edentulous patients after 3 and 6 months since the replacement of their dental prosthesis.

Materials and methods

Patients

All the totally edentulous patients, no wearing dentures for at least 6 months, attending to Outpatient Department of Social Dentistry of our Institute were enrolled: the study has been approved by ethical committee of our Institute (n. RS 321/12).
Criteria for exclusion from the study were antifungal or antibacterial treatment within 30 days of enrollment were excluded from the study as well as patients that had undergone immunosuppressive therapies or were affected by an immunosuppressive disease (diabetes, kidney failure, HIV infection). All patients affected with xerostosmy of idiopathic or iatrogenic origin were also excluded.
A new polymethyl methacrylate dentures were customized for all patients those resulting positive to oral swab were treated with local application of miconazole gel per 7 days and then oral swab were repeated after 10 days from the therapy.
To identify and characterize the different presentations of DRS, we used the Newton Classification described by Budtz-Jorgensen and Bertram (23):
DRS type I – localized inflammation or hyperaemia points (pin point hyperaemia)
DRS type II – diffuse erythema
DRS type III – pseudomembrane formation.

Sample collection and isolation

Before the placement of a new denture, all patients underwent to the examination of the oral cavity. Then, oral swabs were collected from palatal mucosa according to the procedure described by Marcos-Arias et al. (24). All oral swabs were cultured within 2 h from the collection on CHROMagar *Candida* medium (Becton Dickinson GmbH, Germany) and on Sabouraud dextrose agar plates containing chloramphenicol (Becton Dickinson GmbH, Germany) and were incubated at 37° C for 48 h. We considered a *Candida*-associated denture stomatitis to be an isolation of > 10 *Candida* colonies (25).
The plates were scored based on the number of colonies and then subcultured on the same chromogenic medium and Sabouraud dextrose agar to obtain pure cultures.

The oral swabs were repeated in all patient after 6 (time 1) and 12 months (time 2) since the placement of the new dentures.

Characterization of *Candida* species

Isolates were identified by conventional mycological methods such as color formation in CHROMagar *Candida* medium, germ tube tests in calf serum at 37°C for 2 days, and microscopic morphology. Additionally, all yeast identified as *Candida albicans* were screened for their ability to grow at 45°C on Sabouraud dextrose agar for 3 days and for chlamydoconidia formation on Casein agar at 30°C for 10 days (25).

Statistical analysis

For the statistical analysis, SPSS software vers.13 was used (IBM, Armonk, NY, USA). A $p< 0.05$ was considered significant calculated with Chi square test.

Results

We performed 138 oral swabs on edentulous maxillary mucosal in 138 totally edentulous patients. The demographics characteristics of our patients were: 51 (36,9%) female, 89 (64,4%) male with a mean age of 63 years (range 33-88).
At time 0, no patients presented a clinical evidences of stomatitis at the oral examination. Table 1 shows the percentage of swabs positive for the *Candida* spp colonization (41,3%) and the frequency of *Candida* spp isolated based on culture methods.
The most frequently isolated species, at time 0, from oral mucosa were *Candida albicans* 46 (80,70%), followed by *Candida glabrata* 5 (8,77 %), *Candida tropicalis* 1 (1,75%), *Candida krusei* 1 (1,75%) and the simultaneous colonization by *Candida albicans* and *Candida glabrata* 4 (7,01%).
In patients with positive oral swabs (n=57) dentures were replaced only after the local treatment with miconazole gel and the negativization of the maxillary mucosal swabs.
At time 1, oral swabs resulted positive for *Candida* spp were 38 (27,53%) and negative 100 (72,46%).
The most frequently isolated species were *Candida albicans* 19 (50%), *Candida glabrata* 9 (23,68%) and co-colonization *Candida albicans* and *Candida glabrata* in 10 (26,31%) (Table 1).
Among patients with positive oral swabs, the mucosal clinical examination revealed the presence of a denture-stomatitis in 25 patients. In details 17 patients presented of type 1 DRS and 8 patients presented type 2 DRS. Table 2 showed the DRS according to *Candida* spp isolated in the patients with positive oral swabs.
At time 2, oral swabs resulted positive for *Candida* spp were 94 (68,11%) and negative 44 (31,88%). The most frequently isolated species were *Candida albicans* 49 (52,12%), *Candida glabrata* 25 (26,59%) and co-colonization of *Candida albicans* and *Candida glabrata* 20 (21,27%) (Table 1).
Mucosal clinical examination showed signs related to denture stomatitis in 83 patients with oral swabs positive for *Candida* spp.
In particular patients with *Candida albicans* colonization presented 6 type 1 DRS, 35 type 2 DRS and 8 type 3 DRS (Figure 1).
Among patients with *Candida glabrata* oral colonization (n=25), type 1 DRS were found in 14 patient and 11 patients showed no clinical signs of denture stomatitis.
Finally, among patients with simultaneous colonization of mucosa by *Candida albicans* and *Candida glabrata* (n=20), type 1 DRS were found in 3 patients, type 2 DRS in 11 patients and type 3 DRS in 6 patients.
Moreover, 12 patients among those positive to *Candida albicans* colonization at time 0 and receiving the local treatment develop at time 2, in 4 cases *Candida glabrata* DRS and in 8 cases a co-colonization *Candida albicans* and *Candida glabrata* DRS.

Table 1 - Panel A: Number of patients (n=138) with oral swab positive for *Candida* spp at time 0, time 1, and time 2; p<0.05 was considered significative, calculated by two-degree freedom Chi square test.
Panel B: *Candida species* isolated in each of positive oral swabs at time 0, time 1, and time 2; p<0.05 was considered significative, calculated by five-degree freedom Chi square test.

Panel A

	Time 0	Time 1	Time 2	
Positive	57 (41.3%)	38 (27.53%)	94 (68.11%)	p<0.05
Negative	81 (58.69%)	100 (72.46%)	44 (31.88%)	p<0.05

Panel B

Positive Oral Swabs	Time 0	Time 1	Time 2	
Candida albicans	46 (83.63%)	19 (50%)	49 (52.12%)	p=0.05
Candida glabrata	5 (9.09%)	9 (23.68%)	25 (26.59%)	p<0.05
Candida albicans and Candida glabrata	4 (7.27%)	10 (26.31%)	20 (21.27%)	p<0.05

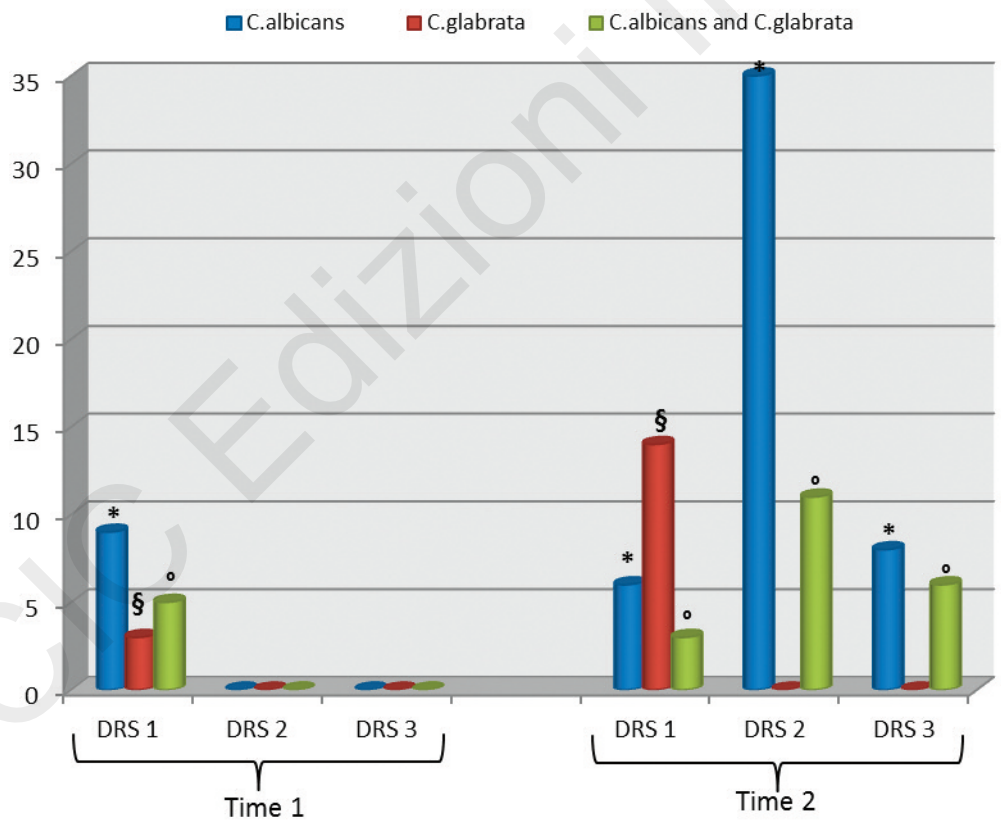

Figure 1
Frequency of patients with positive swab for the different yeast. Differences among the same yeasts for different time and DRS was calculated by chi square test * § ° p< 0.05.

Discussion

Candida spp, especially *Candida albicans*, are frequently commensal yeasts, found in the microbiota of the majority of healthy adults and children. However, these germs can often induce an oral pathology in patients with an impaired salivary function or those not fully immuno-competent. Denture - wearing is a predisposing factor to develop *Candida* – dependent stomatitis in the oral tissues in contact with the prosthesis (26-28). The role of *Candida* as causative agent of stomatitis is confirmed by the finding that this germ is isolated more frequently in oral mucosa of denture – wearing than edentulous individuals.

Our data, at time 0, corroborate the literature data, showing that the superior oral mucosal colonization in total edentulous patients without denture for 6 months was 58%.

Candida albicans remains the main etiological agent, accounting in our series, up to 80% of isolates from oral mucosal epithelium, however our data highlight that *Candida glabrata* has emerged as a notable pathogenic agent of the oral mucosa, either co-infecting with *Candida albicans*, or occurring in single species infections.

Twelve patients with a positive swab for *Candida albicans* at time 0 receiving an antifungal topical therapy developed at time 2 a DRS associated with *Candida glabrata* (4 cases) and *Candida albicans* and *Candida glabrata* DRS (8 cases). This might be explained by an increased susceptibility of *Candida albicans* antifungal therapy compared to *Candida glabrata* or a therapy-induced alteration of local immune response. Another possible explanation for the relatively frequent occurrence of a co-infection of *Candida albicans* and *Candida glabrata* is that these significant differences limit the extent of inter-species competition allowing the organisms to occupy similar oral niches (29, 30).

Our data suggest also an association between *Candida* spp colonization and clinical features of denture stomatitis: in particular is evident how patients who develop a *Candida albicans* DRS tend to have a more severe compared to patients with *Candida glabrata* DRS. This finding may be partially explained considering not only the reported lower pathogenicity of *Candida glabrata* related to its morphological and biochemical properties but also the minor local immune response given by the *Candida glabrata* respect to *Candida albicans* on mucosal epithelial cells (29).

These hypotheses are supported by the fact that when is present a co-colonization by *Candida albicans* and *Candida glabrata* the clinical manifestation of denture stomatitis is more severe with respect to when denture stomatitis was associated with *Candida albicans* or *Candida glabrata* colonization alone.

Our results confirm that the co-colonization with *Candida albicans* and *Candida glabrata* infection is associated with a more severe clinical form of denture stomatitis according to the Newton Classification; it is likely that *Candida glabrata* has some virulence factors that help the *Candida albicans* in invading the oral mucosa and reach the blood vessels. It's well-known data that *Candida glabrata* is the third most common cause of mortality in hospitals (32).

Our data also reveal that even by administering a next generation prosthetic device, the tendency to the development of a DRS is around 70% and how this tends to worsen over time where the graft is taken even if they implemented all precautions to reduce the formation of biofilm. Yeasts and bacteria co-aggregate as biofilms on the fitting surface of the denture rather than on the mucosal surface and have the ability to cause damage to the oral mucosa, which is typified by inflammation and hyperplasia of the denture-loading tissues (4).

Biofilm formation is a key factor in the pathogenesis of *Candida* spp – related stomatitis. In fact, once the biofilm is formed, it limits the penetration of immune molecules that serves for the recognition of the antigen, as well as anti-fungal treatments (29).

Moreover, biofilms formed by *Candida albicans*, non albicans *Candida* like, *Candida glabrata, Candida parapsilosis*, *Candida tropicalis* have been associated with higher morbidity and mortality rates compared with isolates unable to form biofilms and some studies have pointed out that biofilm-forming ability is greater for *non-albicans* species than for *albicans* species (31, 32).

Surely the time factor also plays a role in respect of the mechanical-action carried out by the same prosthesis pressure towards the oral epithelium and the possible role of this factor in altering the local immune balance.

More studies will be needed to investigate at cellular molecular levels the possible role of chronically pressure due to denture on mucosal epithelium and its possible role in oral immune local response.

Conflict of interest

None.

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