

EPIDEMIOLOGY AND PHOSPHOLIPASE ACTIVITY OF ORAL *CANDIDA* SPP. AMONG PATIENTS WITH CENTRAL NERVOUS SYSTEM DISEASES BEFORE AND AFTER DENTAL CLEANING PROCEDURE

Aurélia Silva Ribeiro¹, Dágma Aparecida Silva¹, Francislene Pereira Silva¹, Gleicy Carla Santos¹, Livia Maria Soares Campos¹, Lorena Vivien Neves Oliveira¹, Daniel Assis Santos^{1*}

¹ Laboratório de Microbiologia, Faculdade de Ciências da Saúde, Universidade Vale do Rio Doce, Governador Valadares, MG, Brasil.

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ABSTRACT

Patients suffering of diseases that affect central nervous system may be considered more susceptible to the infectious diseases of mouth. Sixty-nine patients suffering of cerebral palsy, Down's syndrome and metal retardation were submitted to saliva examination for the presence of *Candida* spp. before and after a procedure of dental cleaning. The isolates were submitted to assay for verifying phospholipase production. 55.10% of the patients provided isolation of *Candida* spp. The frequency of isolation obtained before dental procedure was: *C. albicans* (83.33%), *C. krusei* (8.33%) and *C. kefyr*, *C. parapsilosis* and *C. glabrata* (2.78% each). The frequency after the procedure was: *C. albicans* (68.57%), *C. parapsilosis* (11.43%), *C. krusei* and *C. kefyr* (8.57% each) and *Candida glabrata* (2.86%). We verified significantly difference ($p < 0.01$) between populations obtained at the two examinations. Phospholipase production was verified only among *C. albicans* strains and the proportion of producers was higher when testing isolates obtained after dental cleaning procedure. Studies focused on *Candida* spp. isolation are useful for better comprehension of the role of these yeasts on the oral flora from patients with cerebral palsy, Down's syndrome and metal retardation.

Key words: *Candida* spp., cerebral palsy, Down's syndrome, metal retardation, phospholipase.

INTRODUCTION

Cerebral palsy describes a group of permanent disorders of the development of movement and posture, causing activity limitation that are attributed to nonprogressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition,

communication, and behaviour, by epilepsy and by secondary musculoskeletal problems (1). Patients suffering of Down's syndrome, Cerebral palsy and/ or Mental Retardation can present mechanical problems in performing oral hygiene, resulting in high proliferation of oral flora (10).

Candida species, mainly *C. albicans* can live as commensal microorganism in healthy individuals, but it is capable of causing infection if there are predisposing

*Corresponding Author. Mailing address: Laboratório de Microbiologia, Faculdade de Ciências da Saúde, Universidade Vale do Rio Doce, Rua Israel Pinheiro, 2000, Bairro Universitário, 35020-220, Governador Valadares, Minas Gerais, Brasil.; E-mail: danielsantosassis@yahoo.com.br

conditions related to the host. The infective ability of this yeast also depends on the production of several virulence factors such as germ tube, protease and phospholipase and growth at 37°C (7, 9, 15, 19).

Oral colonization with yeast is known to be significantly higher among immunocompromised patients than among healthy people. An increased incidence of asymptomatic oral *Candida* spp. carriage in HIV positive individuals compared with others at risk groups such as denture wearers has also been noted (3).

Previous studies demonstrated that pathoanatomical disorders predispose children with Down's syndrome to the proliferation of *Candida* strains, completely populating the floor of the mouth, often causing candidiasis. The onset of the infectious process by *Candida* in the mouth of children with Down's syndrome, in addition to abnormal innate and acquired immunological conditions, is also influenced by the pathogenic capacity of these yeasts, initially acting as colonizers and later on as infectious agents due to the alteration of the oral flora produced by microbiological, chemical and physical factors, such as chewing and/ or poor mouth cleaning (14, 15).

Considering that little is known about *Candida* spp. on oral flora from patients with Down's syndrome and that studies focused on the isolation of these microorganisms from patients with cerebral palsy and/ or mental retardation are scarce, we aimed to isolate yeasts from the genus *Candida* from people suffering of these diseases. We also analyzed the phospholipase activity and the influence of dental cleaning procedure on the isolation of the yeasts.

PATIENTS AND METHODS

Studied population

Sixty-nine patients with mental retardation (39 individuals), cerebral palsy (19 individuals) and Down's syndrome (11 individuals) aged from 6 to 48 years old, attended by a reference mouth care (Pronto Atendimento Odontológico ao Paciente Especial) from Governador Valadares, Minas Gerais, Brazil, were studied from February to

May of 2008. 60.90% of the patients were male and 39.10% were female. This study was approved by the Ethics Committee of the Universidade Vale do Rio Doce (CEP/UNIVALE 012/2008). In all the patients, saliva was collected by swabbing the oral cavity and from the tongue at two different moments: before and immediately after dental cleaning procedure. No antibacterial and/ or antifungal drugs were used in the patients during the cleaning process, which takes around 30 minutes. No patients presented lesions suggesting oral candidosis.

Mycological examination

Clinical samples were immediately inoculated on Sabouraud-Dextrose Agar (SDA) plates containing chloramphenicol 0.1 g/L and were incubated during 48h at 28°C. The yeasts colonies were subcultured on SDA slants were kept at 4°C for subsequent identification. The isolates obtained were identified by the germ tube test and by the morphological and biochemical profiles (17).

Phospholipase production

Candida strains were screened for production of extracellular phospholipase activity by growing them on egg yolk agar and measuring the size of the zone of precipitation by the method of Price *et al.* (12). Colonies growing for twenty-four hours were subcultured to the egg yolk agar and then incubated at 37°C for 48 h. The diameter of the precipitation zone around the colony was determined. Measurement and calculation of the zone of the phospholipase activity (Pz) were calculated as follows: colony diameter/ colony diameter + precipitation zone. Pz coefficient were classified as negative (Pz = 1.00), positive (0.64 ≥ Pz < 1.00) and strongly positive (Pz < 0.64).

Statistical analysis

The comparison between obtained results was performed by using the Chi-square test. A p value < 0.01 was considered to be significant.

RESULTS AND DISCUSSION

From all the studied individuals, 38 (55.10%) provided the isolation of *Candida* spp.. Sex, age and pathological condition did not provided different results between the isolation from patients with one of the three diseases ($p < 0.01$). This is the first study which involves *Candida* spp. isolation from patients with cerebral palsy and mental retardation. Some works involving Down’s syndrome children were found. Carlsted *et al.* (2) verified that *Candida* spp. colonization in patients with Down’s syndrome were significantly higher than control individuals. These three groups of patients from our study could be considered more susceptible to mouth infectious diseases, including candidosis, because they may suffer from multiple disorders such as orofacial anomalies, open mouth posture, macroglossia, frequent respiratory diseases, immunological deffects and mouth breathing. The incidence of angular cheilitis (angular stomatitis, perleche and commissural fissures) also appears to be increased (14). According to Vieira *et al.* (18), *Candida* spp. from the mouth mucosa from patients

with Down’s syndrome could take part of dental biofilm in a high proportion, concomitantly with *Streptococcus mutans*, probably influencing the occurrence of caries, gingivitis and periodontitis.

Table 1 demonstrates the isolation of *Candida* spp. at the different moments of saliva obtaining. Eighteen patients propitiated *C. albicans* isolation at the two times of examination. Considering isolation before dental procedure, 36 strains were obtained from 34 patients, being *C. albicans* obtained from 30 patients (83.33%), followed by *C. krusei* from 3 (8.33%), *C. kefyf*, *C. parapsilosis* and *C. glabrata* from 1 patient each (2.78% each). After dental procedure, *Candida* isolates were obtained from 32 patients, who provided 35 strains distributed as: *C. albicans* from 24 patients (68.57%), followed *C. parapsilosis* from 4 patients (11.43%), *C. krusei* and *C. kefyf* from 3 patients each (8.57%) and *Candida glabrata* from 1 patient (2.86%). We verified significantly difference ($p < 0.01$) between populations obtained in the two studied moments.

Table 1. *Candida* spp. isolates obtained from patients with cerebral palsy, Down’s syndrome and mental retardation before and after dental cleaning procedure

Number of patients	Isolate obtained before dental cleaning procedure	Isolate obtained after dental cleaning procedure
18 (47.37%)	<i>C. albicans</i>	<i>C. albicans</i>
5 (13.16%)	<i>C. albicans</i>	No isolate
3 (7.90%)	No isolate	<i>C. albicans</i>
1 (2.63%)	<i>C. albicans</i>	<i>C. krusei</i>
1 (2.63%)	<i>C. krusei</i>	No isolate
2 (5.27%)	<i>C. albicans</i>	<i>C. parapsilosis</i>
1 (2.63%)	<i>C. albicans</i>	<i>C. kefyf</i>
1 (2.63%)	<i>C. glabrata</i>	<i>C. glabrata</i>
1 (2.63%)	No isolate	<i>C. parapsilosis</i>
1 (2.63%)	<i>C. albicans</i> and <i>C. krusei</i>	<i>C. krusei</i>
1 (2.63%)	<i>C. krusei</i>	<i>C. krusei</i> and <i>C. kefyf</i>
1 (2.63%)	<i>C. kefyf</i>	<i>C. kefyf</i> and <i>C. albicans</i>
1 (2.63%)	<i>C. albicans</i> and <i>C. parapsilosis</i>	<i>C. albicans</i> and <i>C. parapsilosis</i>
1 (2.63%)	<i>C. albicans</i>	<i>C. albicans</i> and <i>C. parapsilosis</i>

The higher prevalence of *C. albicans* attested previous studies focused on the isolation of yeasts from mouth. Furthermore, this species was the most prevalent at the two tested moments, although the frequency was reduced ($p < 0.01$) from 83.33% to 68.57% when comparing isolates obtained before and after dental procedure. In addition, non *albicans* species (*C. parapsilosis*, *C. krusei* and *C. kefyr*) were more frequently ($p < 0.01$) after the treatment. *C. tropicalis* was not isolated from the patients of this study, differing from other researchers that obtained this species as the second most common in oral cavity (13). Erköse and Erturan (3) found that *C. albicans* was the most frequent isolated from patients infected with human immunodeficiency virus, corresponding to 82.8% of the studied patients. On the other hand, Resende *et al.* (13) and Oksuz *et al.* (11) obtained *C. albicans* isolation from 62.66% and 66.4% of the patients from their works. These data are similar to that found in our work. It is interesting to observe the increasing on the occurrence of *C. parapsilosis* at the second moment of mouth examination. This pathogen has been mentioned as an emergent pathogen and has been isolated in high frequency by other researchers (4).

Two patients provided isolation of two different isolates concomitantly before dental procedure: *C. albicans* and *C. krusei*, which provided only *C. krusei* after the procedure; *C. albicans* and *C. parapsilosis*, which were maintained after dental procedure. Four patients presented two species concomitantly after the procedure, but only one was preceded by the same isolation (this was the patient mentioned above); the other isolations were: *C. krusei* and *C. kefyr*; *C. kefyr* and *C. albicans*; *C. albicans* and *C. parapsilosis* (Table 1). All of them were preceded by the isolation of only one strain: *C. krusei*, *C. kefyr* and *C. albicans*, respectively. The occurrence of more than one *Candida* spp. isolate from the same patient was previously demonstrated by Erköse and Erturan (3). It is possible to presume that all these patients were colonized by the two species isolated at both examination times; being the dental procedure responsible for selecting the strain (s) that was more able to adhering to mouth mucosa and it would be isolated. It is also plausible that if only one strain was obtained

before dental cleaning and two strains were obtained after that, this procedure could be reducing the population of the species isolated previous (probably the most virulent), propitiating the isolation of two different microorganisms, being the second one possible less virulent.

Six patients presented positive isolation only before the dental cleaning process (from whom 5 were *C. albicans*) and four isolation only after the procedure (from whom 3 were *C. albicans*). From our point of view, the isolation of *C. albicans* only after dental procedure could be attributed to a possible competition between yeasts and bacteria at the moment of the saliva obtaining. It may well yield the result that the microbiological examination tends to isolate the strain that would be pathogenically predominant at the time.

The phospholipase production was verified to be positive only among *C. albicans* isolates, being the other species negative for its production. 15 (50.00%) and 14 (58.33%) *C. albicans* were phospholipase producers before and after dental procedure, respectively. These results are similar to that found by Gokce *et al.* (5), who verified phospholipase production only in *C. albicans*. When studying the phospholipase production from *Candida* spp. isolated from anatomically distinct sites of healthy adults, Oksuz *et al.* (11) verified that the activity of exoenzyme was higher in oral isolates. The presumed mechanism for all invasive *C. albicans* disease involves initial mucosal surface colonization followed by invasion into the adjacent tissues and organs. The ability of *C. albicans* to produce secretory enzymes such as phospholipase may play an important role in the pathogenicity of these yeasts (10, 15, 16, 19). According to Ibrahim *et al.* (6), extracellular phospholipases facilitate the ability of these organisms to injure, invade, egress from various host cells and/or remove processed antigens from the surface of antigen-presenting cells. We verified an increasing of phospholipase production from isolates obtained after dental procedure. Probably it may act as an advantage factor for the colonization and persistence of the yeasts in oral mucosa. Furthermore, commensal organisms such as *C. albicans* are able to persistently colonize the host without causing symptoms. However, the balance between

commensalism and pathogenicity is delicate (8). How these two states are modulated during colonization is a major area of research in medical mycology, with the aim of utilizing the knowledge gained for the benefit of the patient (19). Considering that patients from our study may be more susceptible for mouth infectious diseases, further studies are necessary to clarify whether *Candida* spp. population in oral mucosa could constitute a risk factor for candidosis and whether dental cleaning procedure could be a preventive action for that diseases.

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