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ORIGINAL

Prevalence of yeast species in the oral mucosa of patients with periodontal disease in the menopausal stage

Prevalencia de especies de levaduras en la mucosa bucal de pacientes con enfermedad periodontal en etapa menopáusica

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ABSTRACT

Introduction: substantial, transient, physiological or pathological endocrine changes can affect periodontal condition through modulation of the inflammatory response, as well as through a direct action on periodontal pathogens.
Objective: to determine the prevalence of yeast species in the oral mucosa of patients in the menopausal stage with periodontal disease.
Methods: observational, descriptive, cross-sectional study. Patients who attended the dental consultation, within the framework of the private practice of general dentistry, in the Autonomous City of Buenos Aires, between March and August 2022, who were diagnosed with periodontal disease, were evaluated, which allowed obtaining a sample of 25 female patients from a universe of 50, selected from the exclusion and inclusion criteria and a simple random sampling. For statistical analysis of the data obtained, the epi-info programs and the GNU pspp version 1.4.1 and JASP 0.16.4 programs were used.
Results: In 14 positive cases of yeast colonization in mucosa, 12 had positive colonization in the sulci and 2 were negative. Of the remaining 11 cases that had negative results in the mucosa, all were negative in the sulcus. No case was found in which the result was negative in the mucosa and positive in the sulcus. Oral examination revealed that 52 % of the patients had stage 2 periodontal disease and as for stages 1 and 3, 24 % of the patients were in this range.
Conclusions: Candida albicans was the predominant species both in the oral mucosa and in the periodontal sulcus in postmenopausal patients. In both studied sites, buccal mucosa and periodontal sulcus, other opportunistic species such as C. parapsilosis, C. kruzei, C. tropicalis and C. glabrata were also found.

Keywords: Buccal Mucosa; Menopause; Periodontal Disease; Bacteria; Candida Albicans.

RESUMEN

Introducción: los cambios endocrinos sustanciales, transitorios, fisiológicos o patológicos, pueden afectar la condición periodontal a través de la modulación de la respuesta inflamatoria, así como a través de una acción directa sobre los patógenos periodontales.
Objetivo: determinar la prevalencia de especies de levaduras en la mucosa bucal de pacientes en etapa menopáusica con enfermedad periodontal.
Métodos: estudio de observacional, descriptivo de corte transversal Se evaluaron las pacientes que concurrieron a la consulta odontológica, dentro del marco de la práctica privada de odontología general, en la Ciudad Autónoma de Buenos Aires, entre marzo y agosto de 2022, a las cuales se les diagnosticó enfermedad periodontal, lo que permitió obtener una muestra de 25 pacientes de sexo femenino de un universo de 50, seleccionadas a partir de los criterios de exclusión e inclusión y un muestreo aleatorio simple. Para el análisis estadístico de los datos obtenidos, se utilizaron los programas epi-info y GNU pspp versión 1.4.1 y JASP 0.16.4.
Resultados: en 14 casos positivos de colonización de levaduras en mucosa, 12 tuvieron colonización positiva en los surcos y 2 fueron negativos. De los 11 casos restantes que tuvieron resultados negativos, en las mucosas todos fueron negativos en surcos. No se encontró ningún caso en que tuviera resultado negativo en mucosa y positivo en surco. Al examen bucal se pudo detectar que el 52 % de los pacientes presentaba enfermedad periodontal en estadio 2 y en cuanto a los estadios 1 y 3, el 24 % de los pacientes estaban en este rango.
Conclusiones: Candida albicans fue la especie predominante tanto en mucosa bucal como en el surco periodontal en pacientes postmenopáusicas. En ambos sitios estudiados, mucosa bucal y surco periodontal también fueron encontradas otras especies oportunistas como C. parapsilosis, C. kruzei, C. tropicalis y C. glabrata.

Palabras clave: Mucosa Bucal; Menopausia; Enfermedad Periodontal; Bacterias; Candida Albicans.

INTRODUCTION

Periodontitis is one of the first human diseases recognized to be associated with mixed-species biofilms. This biofilm contains many microorganisms, including bacteria, fungi, and viruses. Several studies have reported increased subgingival colonization by yeasts, particularly C. albicans, in patients with chronic periodontitis compared to periodontally healthy subjects.1,2

Several Candida species are recovered from the human oral cavity, including C. albicans, C. glabrata, C. Kruse, C. tropicalis, C. parapsilosis, and C. guilliermondii. The most common fungal infection of the oral mucosa is candidiasis, mainly caused by C. albicans species, a normal commensal of the oral cavity, behaving as an opportunistic pathogen. The oral mucosa constitutes the main reservoir of Candida spp, usually on the surface of the oral cavity's tongue and vestibular and lingual mucosa. The proportion of C. albicans in the oral yeast population can be as high as 50-80 %.1,3

While there is no doubt that biofilm is the cause of periodontal disease, its expression results from the interaction of bacterial, host, environmental, and systemic factors. This interaction leads to the individuality of disease expression, which, in turn, leads to the individuality of treatment. Including fungi as commensals in the oral biofilm is an important and innovative concept in oral biology. Thousands of bacterial phylotypes and more than one hundred fungi can colonize the oral cavity. Taxonomic profiling combined with functional expression analysis has revealed that Candida albicans, Streptococcus mutans,

and prominent periodontopathogens are not always present or numerically important in candidiasis, caries, or periodontitis lesions.1

However, C. albicans combined with Streptococcus spp increase their virulence in invasive candidiasis, early childhood caries, or peri-implantitis. Recent studies revealed that not only periodontopathic bacteria but also herpes viruses, e.g., Herpes simplex virus 1, Epstein-Barr virus, human cytomegalovirus, and oral C. albicans are involved in periodontitis. C. albicans can be detected in subgingival sites of patients with periodontitis, indicating that C. albicans can colonize inflammatory periodontal pockets and is significantly implicated in periodontitis.4-6 Several studies report the presence of Candida spp in subgingival tissues from 10 to 30% in healthy patients, being even higher in immunocompromised and diabetic patients.7

Normal periodontal aging results from cellular aging, the basis for the intrinsic changes observed in oral tissues over time. The aging process does not affect all tissues in the same way. Epithelial tissue, one of the periodontium's main components, is always being renewed. In the epithelium, a progenitor population of cells (i.e., stem cells) in the basal layer provides new cells.4

Basal layer cells are the least differentiated cells of the buccal epithelium. A small subpopulation of these cells produces basal cells and retains the proliferative potential of the tissue. A larger subpopulation of these cells (amplifying) produces cells that are available for further maturation. This maturing cell population continually undergoes a process of differentiation or maturation. By definition, the differentiated cell (the epithelial cell) can no longer divide. In contrast, the basal cell remains part of the progenitor population of cells ready to return to the mitotic cycle to produce both cell types again. There is a constant source of renewal.7

Substantial endocrine changes, transient, physiologic, or pathologic, can affect the periodontal condition through modulation of the inflammatory response and direct action on periodontal pathogens. Periodontal manifestations of sex steroid hormones can be observed in endocrine changes that occur during puberty, the menstrual cycle, pregnancy, and menopause. The endocrine and reproductive systems control these changes.8,9

The totality of epidemiological studies shows that the increased prevalence and severity of gingivitis and periodontitis are closely associated with poor oral hygiene. Moreover, the imbalance between bone formation and resorption created by estrogen deficiency may manifest itself early in the alveolar structure by creating negative bone volume, which increases the risk of periodontal disease and tooth loss.10

For this reason, the association between the endocrine system and periodontal diseases has been specifically addressed in the current classification system, emphasizing its importance in diagnosis and treatment. Hence, the importance of focusing research on the prevalence of yeasts in the oral mucosa of patients with periodontal disease in the menopausal stage.

METHODS

An observational, descriptive, cross-sectional, descriptive study was carried out. The patients who attended the dental office, within the framework of the private practice of general dentistry, in the Autonomous City of Buenos Aires between March and August 2022, who were diagnosed with periodontal disease, were evaluated, which allowed obtaining a sample of 25 female patients out of a universe of 50, selected based on the exclusion and inclusion criteria and a simple random sampling.

Inclusion criteria

- Female patients, immunocompetent, non-smokers.
- Age 48 years and older
- In peri-, menopausal or post-menopausal stage, not receiving hormone replacement therapy.
- With a diagnosis of periodontal pathology stage II and above.

Exclusion criteria

- Were receiving hormone replacement therapy (5 patients).
- Were receiving systemic antifungal or antimicrobial treatment in the last 3 months, the majority of which were patients being treated for onychomycosis (5 patients).
- They did not wish to participate in the study (3 patients).
- Suffered from gingivitis but not periodontitis (8 patients).
- In periodontal and gingival health (4 patients).
- Patients who did not give their consent to participate in the study after having been duly informed.

Once the patients were selected, the following procedures were performed:

- Signing of informed consent.
- Update of general medical history.
- Clinical examination of soft and dental tissues.
- Collection of microbiological samples.
- Measurement of periodontal sulcus depths and bleeding on probing.
- Hygiene instructions and diagnostic report for treatment in cases that need it.

Each patient was given a medical history with personal and family history; the odontography was completed according to clinical review and the measurement of clinical indicators with a pressure-controlled periodontal probe:

Probing depth (PS) was performed on all teeth on their four sides: mesial, vestibular, distal, and palatal/lingual. The probing depth was considered normal up to 3 mm around the teeth, and those teeth with the highest PS were considered for evaluation.

Collection of buccal mucosa samples

Before collecting clinical material, the patient read and signed the informed consent form (Appendix I). Each patient was asked to perform a mouth rinse with sterile distilled water. Then, the cheeks, tongue, and palate were swabbed with sterile swabs. They were stored separately labeled in Eppendorf tubes with 100 ml of sterile distilled water. The materials and instruments used for this study were sterilized with proton level as advised by the World Health Organization (WHO) in an autoclave at 134°C during 18 minutes of a plateau in the office where the samples were taken, supervised by Kims and Integron brand test strips.

Collection of periodontal sulcus samples

To take the samples, the supragingival biofilm was removed with a Hu-Friedy periodontal curette; the tooth surface was rubbed with sterile gauze; the area was isolated with sterile cotton rolls, and a high-powered doctor was used. Subgingival biofilm was collected from the sulcus with a Hu-Friedy curette in the periodontal pocket of 4mm or more, choosing those teeth and faces with greater probing depth. The subgingival biofilm sample was placed in an Eppendorf tube with a sterile physiological solution to perform routine microbiological studies.

With a sterile swab, a sample was taken from the oral soft tissues and placed in another Eppendorf tube conditioned as the previous one, to which the same routine microbiological studies as the previous tube were performed.

The samples were identified with a number given to each patient and recorded in the periodontal record of each one, mentioning the sextant in which the sample was taken.

Sample processing

Conventional mycological study

To carry out objective 2.1: "To demonstrate the prevalence of yeast species in subgingival biofilm and different sites of the oral mucosa (tongue, cartilage, and palate) in peri- and post-menopausal patients with periodontal disease," samples were processed as follows:

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Direct examination and culture

Fresh microscopic studies were performed (Fig. 4), Gram-Nicolle and Giemsa staining, and they were seeded on differential chromogenic solid media (CHROMagar Company, Paris, France), which were also used in other studies and seemed appropriate for comparison purposes (76, 77, 78). They were incubated at 37°C for one week and observed daily for the presence of development.

Identification of isolates

Isolates were identified based on the color developed in the chromogenic medium, observing the presence of one or more species, micromorphology in 1% milk agar-Tween 80 (103) and carbohydrate assimilation profile by API ID 32D commercial systems (®Bio Mérieux, France). The yeasts obtained were grown under the same conditions as the primary culture on Sabouraud agar to obtain pure isolates for identification. They were preserved in water with glycerin (20 %) at -20°C and -70°C.

Data processing and statistical analysis

The results were dumped into tables in the Calc program of the LibreOffice office suite for processing. The programs epi-info and the GNU pspp version 1.4.1 and JASP 0.16.4 were used for the statistical analysis of the data obtained. These were estimated to be at a confidence level of 95% using EpiInfo's StatCalc program.

We complied with the provisions of Law 25326 (Personal Data Protection), which guarantees adequate dissociation of the data and prevents identification of the subjects. In addition, the subjects were conveniently informed in the survey about the scope of the research, considering their participation and signature of the informed consent form as acceptance.

RESULTS

Two of the patients were in the perimenopausal stage, not having menstruated for more than 3 months but less than 12 months. The average number of teeth in the mouth was 18.36 against the number of 32 teeth in the mouth of an adult, i.e., 57 % of the teeth were present in the mouth. Thirteen percent of the patients were smokers, and 16% were ex-smokers who had stopped smoking at least 2 years ago. The 25 patients studied had 12 implants installed, the average number of teeth with fixed prostheses was 14.59, and 56 % used removable prostheses, although 2 of them said they did not use them for eating. (Table 1)

Table 1. Distribution of the sample according to the clinical characteristics studied.

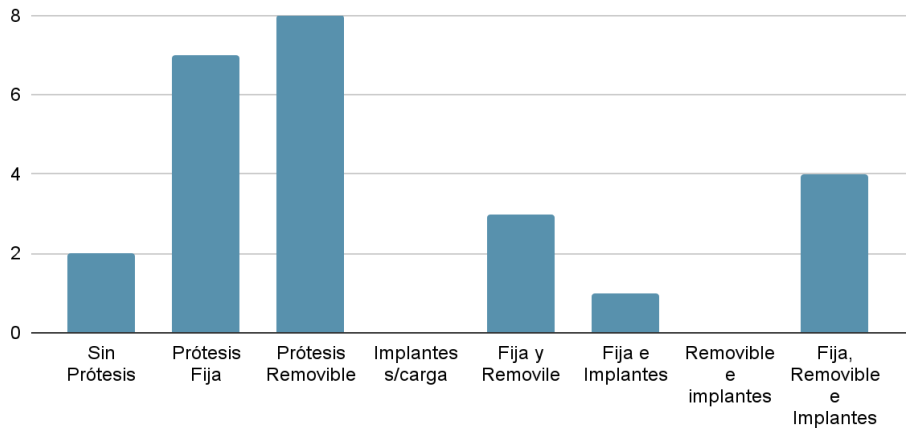
Clinical Characteristics of the Patients Studied		
Age	65,64	Average
Smoking	13 %	Percentage
Missing teeth	341	Absolute value
	13,64	Average
Implants present	12	Absolute values

Patients with fixed prosthesis (alone or in combination with removable prosthesis)	14
Removable Prosthesis Wearers only	8
Removable Prosthesis Carrier Cobalt Chrome (without combination with acrylic prosthesis)	7

Source: own elaboration.

Regarding the presence of prosthetic restorations in the sample studied, 8 patients had removable prostheses, 7 had fixed prostheses and 2 patients did not use any (Figure 1)

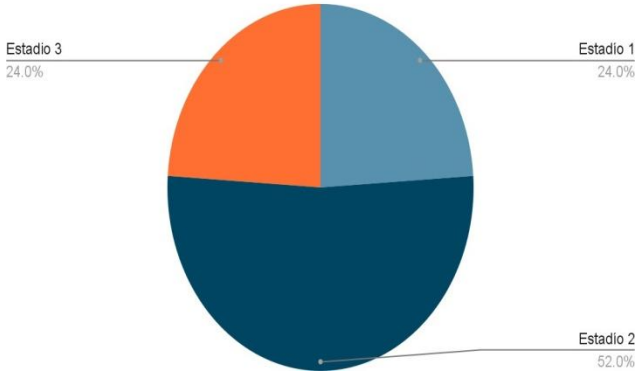
Figure 1. Use of prostheses in the studied sample



Source: own elaboration.

The oral examination revealed that 52% of the patients had stage 2 periodontal disease, and 24% of the patients were in this range for stages 1 and 3 (Figure 2)

Figure 2. Distribution of the sample according to the stages of periodontal disease.



Source: own elaboration.

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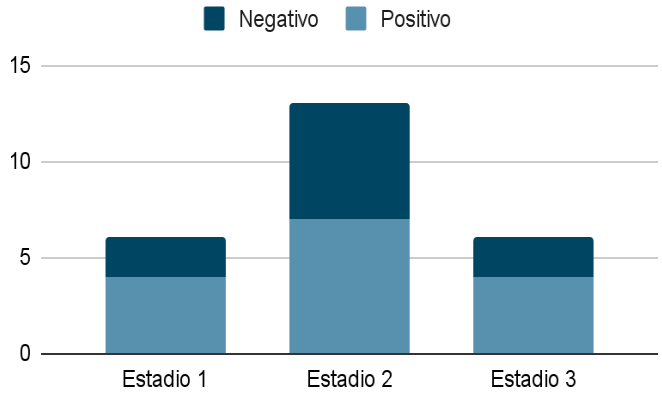
From the mycological studies, all the samples obtained were processed, optical microscopic observations were made of the fresh specimens, and stains were used to recognize the presence of yeasts and some particular characteristics, such as pseudomycelia. To analyze the positive and negative cultures according to the stage of periodontal disease, the guidelines of the new classification of periodontal and peri-implant diseases were considered. Three groups were formed with the stages in which the progression of the patients' pathology was classified. One group in stage 1 (6 cases), one in stage 2 (13 cases), and one in stage 3 (6 cases). (Figure 3)

Figure 3. Positive and negative cultures according to periodontal disease stage.



Source: own elaboration.

In the cases studied (N=25), each patient's positive and negative cultures of mucosa and periodontal sulci were related. In 14 positive cases of yeast colonization in the mucosa, 12 had positive colonization in the sulci, and two were negative. Of the remaining 11 cases with negative mucosal results, all were negative in sulci. No cases were found to be mucosa-negative or sulcus-positive. These results suggest a direct implication between the positive presence of yeasts in the mucosa and their presence in periodontal sulci. (Table 2)

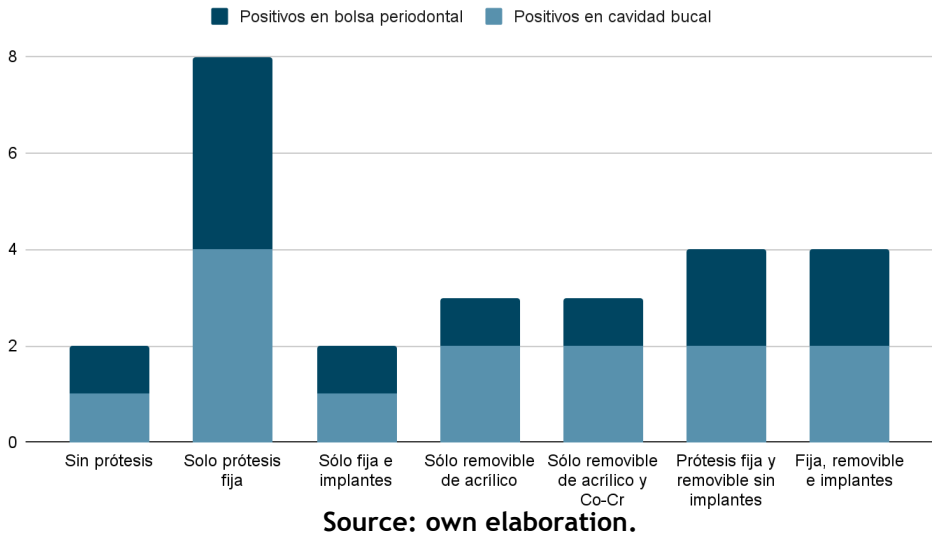
Table 2. Relación de cultivos positivos y negativos en surco y en mucosa

Mucosal culture	Periodontal sulcus culture	Cases
Negative	Positive	0
Positive	Negative	2
Negative	Negative	11
Positive	Positive	12
Total Patients		**25**

Source: own elaboration.

Among the patients who use removable prostheses, we can differentiate between those who use complete prostheses and those who use partial prostheses, and among the latter, between those who use acrylic partial prostheses and those who use cobalt-chrome partial prostheses. None of the patients studied used flexible prostheses, and none were broken. None of the patients studied used removable prostheses on implants. Two (2) patients wore a bruxism plate, and two others have it but do not use it or only use it sporadically. (Figure 4)

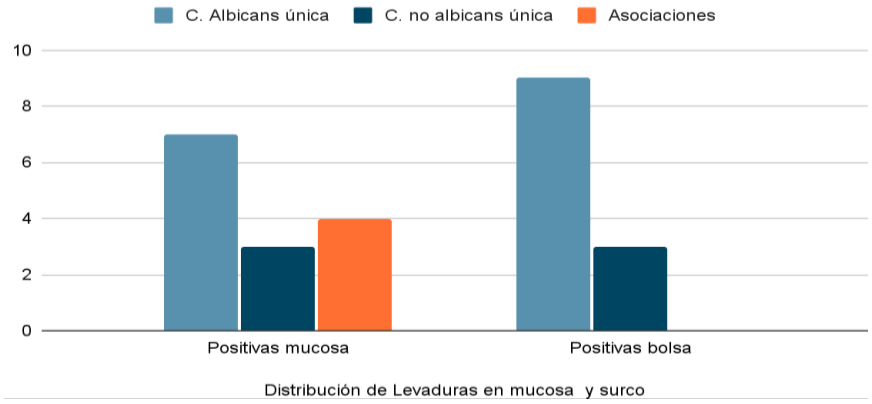
Figure 4. Positive and negative results according to type of prosthesis.



Source: own elaboration.

The associations of yeasts found in periodontal sulci were also analyzed. Of the 25 sites, 13 were negative for yeast colonization (52 %). Of the remaining 12 positives, all cases were colonizations as single species. (Figure 5)

Figure 5. Distribution of yeasts in patients with positive results.



Source: own elaboration.

The difference between the average ages of the two groups was less than 10 (70 years for the positives, with an ES of 2.64 and 77 for the negatives, with an ES of 4.36). The group of refusals had one patient

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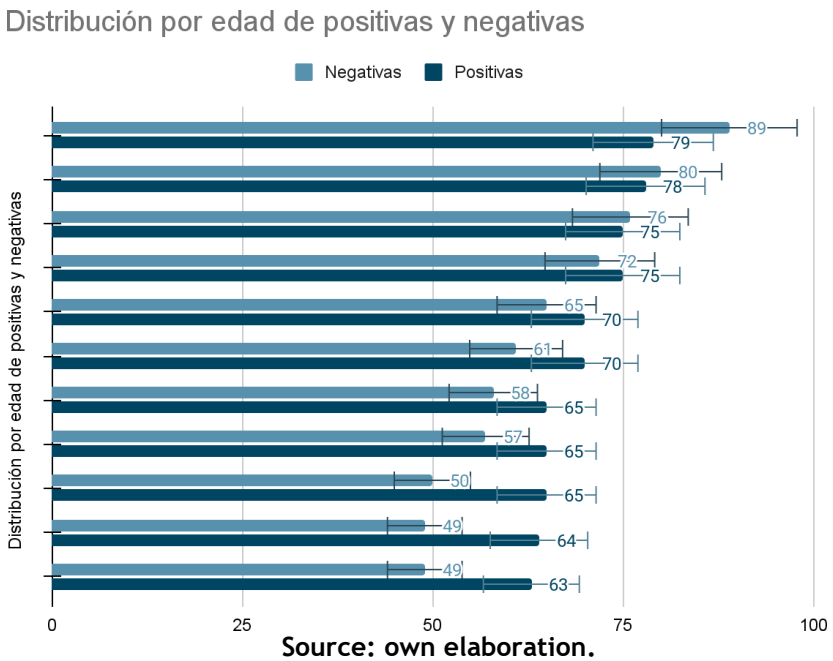
aged 89 years and two aged 49 years. If we remove these two extremes that are not represented in the group of positives, the average age of the patients with negative results from 50 to 79 years old is 64.87 years, with an ES of 3.09. We believe that these variations in standard error are due to the size of the sample, and it would be interesting to repeat this study with a larger sample of patients (Figure 6).

Figure 6. Age distribution of positives and negatives.



Source: own elaboration.

Comparison of the results found for the n=25 population of periodontal patients in menopausal stage with respect to the young periodontal population gave an OR of 2.1891 which indicated the presence of Candida spp is more frequent in menopausal patients than in young patients. (Table 3)

Table 3. Comparison with young patients.

	Menopausal	Youth	Totales
Positive Candida	14	25	39
Negative Candida	11	43	54
Total	25	68	93

Source: own elaboration.

CONCLUSIONS

Candida albicans was the predominant species in both buccal mucosa and periodontal sulcus in postmenopausal patients. Other opportunistic species such as *C. parapsilosis*, *C. kruzei*, *C. tropicalis* and *C. glabrata* were also found in both sites studied, buccal mucosa and periodontal sulcus.

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FINANCING
None.

CONFLICT OF INTEREST
None.