UDK: 6716.311-022.822.8:616.314.17-008.1

ASSOCIATION BETWEEN CANDIDA SPECIES AND PERIODONTITIS

АСОЦИЈАЦИЈА ПОМЕЃУ КАНДИДИЈАЗНИ ВИДОВИ И ПАРОДОНТОПАТИЈА

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Abstract

The mycobiome from the oral cavity is not well characterized, particularly in relation to oral diseases such as periodontal disease. **Aim:** Analysis of the composition of the yeast microbiota present in the oral mucosa and subgingival sites of healthy individuals without periodontitis (HI), and patient with chronic periodontitis (CP), and assessment of the relationship between the severity of this condition and the Candida infection. **Material and methods:** Microbiological samples were obtained from 30 patients (25 and 62 years; 41.33±5.54), divided into 3 groups: 10 patients without CP (control), 10 with moderate CP, and 10 with severe CP. Oral swabs samples were collected from tongue and buccal mucosa with sterile cotton stick. The subgingival samples were obtained from gingival sulcus (HI) or deepest probing depths (patients with CP) by means of a sterile curette (S4L/4R SS). Swab cultures were immediately inoculated on CHROM agar and incubated at 37°C for 48 h. C.albicans and Non-albicans Candida, were identified by the morphology and pigmentation of the colonies. The results were processed by the method of descriptive statistics. **Results:** The percentage of yeast carriers in mucosa in the three groups was similar. Patients with chronic periodontitis showed significant differences in subgingival colonization compared to HI (45% severe, 30% moderate, versus 14,3% of HI). C. albicans was the most common species in the examined patients. **Conclusion:** Subgingival colonization by yeasts could be favored in chronic periodontal disease. **Key words:** Chronic periodontitis, C. albicans, Non-albicans Candida, periodontal pocket.

Апстракт

Микобиомот во усната празнина не е сосема дефиниран, особено во однос на оралните болести како што е пародонтопатија. **Цел:** Анализа на составот на габичниот микробиом присутен на оралната мукоза и субгингивалните регии, кај здрави лица без пародонтална болест (ЗП) и пациенти со хронична пародонтопатија (ХП), и одредување на поврзаноста на стадиумот на заболувањето со кандидната инфекција. **Материјал и метод:** Микробиолошките примероци беа земени од 30 пациенти (25 и 62 години; 41,33 ± 5,54), поделени во 3 групи: 10 испитаници без ХП (контрола), 10 со умерена форма на ХП и 10 со напредната ХП. Оралните брисеви беа земени од јазик и букална лигавица, со стерилно памучно стапче. Субгингивалните примероци беа земени од гингивалниот сулкус (ЗП) или од пародонталните џепови со најголема длабочина (пациенти со ХП) со помош на стерилна кирета (S4L/4R SS). Културите со брис беа инокулирани на подлога агар CHROM и инкубирани на 37 °C за време од 48 часа. Инокулатите од C.albicans и Non-albicans Candida беа идентификувани со морфологија и пигментација на колониите. Резултатите беа обработени со метод на дескриптивна статистика. **Резултати:** Процентот на мукозната габична инфекција кај сите три групи беше сличен. Кај испитаниците со ХП постојат значителни разлики во субгингивалната колонизација во однос на ЗП (45% напредната форма, 30% умерена, 14,3% ЗП). C.albicans беше најчестиот вид во однос на останатите специеси кај испитаниците. **Заклучок:** Субгингивалната колонизација на габи може да биде фаворизирана кај хроничната пародонтопатија. **Клучни зборови:** Хронична пародонтопатија, C. albicans, Non-albicans Candida пародонтален џеп.

Introduction

Periodontitis is a chronic inflammatory disease characterized by destruction of support connective tissue and alveolar bone loss with formation of a periodontal pocket[1]. Results of epidemiological studies have shown that chronic periodontitis (CP) has both a high prevalence and severity in the world and is the most common cause of tooth loss worldwide[2].

Periodontitis has a polymicrobial character; dental plaque with pathogens such as Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans and Tannerella forsythia initiating the disruption of tissue homeostasis[3, 4]. Besides periodontopathogens, dental

plaque contains other bacteria as well as fungi and viruses whose role is actively studied. The high compliance of C. albicans allows it to colonize indifferent media creating mixed biofilms with commensal as well as pathogenic bacteria in aerobic and anaerobic conditions, which makes C.albicans an active participant in the inflammatory-destructive process in periodontal diseases. Many researchers consider that yeast-like fungi, specifically Candida spp., are one of the important causes for development, progression, and complicated course of CP[5].

Candida species are commensal yeasts, around 40% of healthy people carry members of the genus Candida in saliva or oral mucosa[6]. Certain local and/or general predisposing factors can increase its invasion in mucosal tissues and cause opportunistic infections. It occurs usually in immunocompromised individuals with endocrinal disorders, blood diseases, and with long-term use of broad-spectrum antibiotic therapy.

C. albicans is the most prevalent yeast of oral microbiota. It constitutes 60% to 70% of total isolates of this genus, but other Candida species including Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida krusei, Candida dubliniensis, Candida glabrata, are also found[7].

Candida species typically reside on the tongue, palate, buccal mucosa, saliva, but also in other oral sites such as pulp chamber, carious lesions and periodontal pockets. The periodontal pocket and gingival crevicular fluid are favorable media for C. albicans germination and hyphal tip growth.

Several studies have reported increased subgingival colonization by yeasts, particularly Candida albicans, in chronic periodontitis patients compared to periodontally healthy subjects[8,9,10]. Furthermore, it has been reported that the proportion of yeasts in periodontal pockets is similar to some bacterial periodontopathogens, suggesting a possible role for Candida spp. in the pathogenesis of the disease[11,12,13].

Aim of the study

The aim of the study was to analyze the composition of the yeast microbiota present in the oral mucosa and subgingival sites of healthy individuals without periodontitis (HI), and patient with chronic periodontitis (CP), and to assess the relationship between the severity of this condition and the Candida infection.

Material and method

Study Population and Clinical Examination

This prospective study randomly recruited 60 patients that attended a check-up or review appointment at the Faculty of Dentistry, Ss. Cyril and Methodius University in Skopje, and the University Dental Clinical Center "St. Pantelejmon" in Skopje, Republic of North Macedonia. Study sample consisted of 38 females (63,3%) and 22 males (36,6%) with a mean age of 48.2 years (age range between 30 and 62 years; 41.33±5.54). Individuals were notified of the nature of the study, by signing informed consent forms. All the subjects fulfilled the following general criteria; no history of systemic diseases, pregnancy, immunosuppression, antibiotic treatment, antimycotic and anti-inflammatory drugs in the 6 months prior to the study, previous periodontal treatment, use of orthodontic appliances, use of partial and/or total prosthesis and presence of local and/or systemic factors that predispose candidiasis.

Periodontal Examination

The assessed clinical variables were: plaque index (PI)[14], probing pocket depth (PPD), and clinical attachment loss (CAL). Probing pocket depth and attachment loss were measured using a standard periodontal probe, at six sites per tooth, i.e., distobuccal, buccal, mesiobuccal, distolingual, lingual, and mesiolingual, in all teeth, excluding third molars. A set of full-mouth standardized intraoral radiographs was obtained from each patient. The clinical diagnosis based on the classification of the American Academy of Periodontology (AAP)[15], was established according to clinical parameters and radiograph information. Patients were classified according to diagnosis and severity of Chronic periodontitis (CP) based on the probing pocket depth (PPD) and the CAL index (Clinical Attachment Loss), measured as a distance in mm from the cementoenamel junction to the bottom of the periodontal pocket, in 30% of the teeth; (American Academy of Periodontology 1999). Periodontitis is defined as the presence of clinical attachment loss in at least 2 nonadjacent teeth or the presence of buccal or oral clinical attachment loss ≥3 mm with pocketing >3 mm in at least 2 teeth[15]. Stages disease were defined according to severity of periodontal breakdown and the following criteria: Stage I, initial periodontitis where CAL at the site of greatest loss was 1 to 2 mm and maximum probing depth was 4 mm; Stage II, moderate periodontitis in which CAL at the site of greatest loss was 3 to 4 mm and maximum probing depth was 5 mm; Stage III, severe periodontitis with CAL at the site of greatest loss was ≥5 mm and probing depth was ≥6 mm. Based on these indexes, the patients were divided into 3 groups:

A. **Control group (CG)**: 20 periodontally healthy subjects;
B. **Moderate Chronic Periodontitis group (MCP)**: 20 patients with moderate chronic periodontitis;
C. **Severe Chronic Periodontitis group (SCP)**: 20 patients with severe chronic periodontitis (SCP).

Clinical specimens

Following full-mouth periodontal examination, two oral samples were collected for the microbiological study, one from the tongue and buccal mucosa and one from the gingival sulcus/periodontal pocket. The samples of oral mucosa were collected before the subgingival plaque sample, from mucosa of both cheeks and from the third half of the dorsal side of the tongue using small sterile cotton swab.

For each patient with periodontitis (PD≥ 3 mm), three to five samples were collected from the deepest probing site, while for PH subjects, subgingival plaque samples were collected from the gingival sulcus (PD ≤ 3 mm), by means of a sterile Gracey curette (S4L/4R SS). Swab cultures were immediately inoculated on chromogenic medium- (CHROMagar Candida) and incubated at 37°C for 48 hours (Biobase Constant-Temperature Incubator, BJPX-H30II) in the biochemical laboratory at the Faculty of Dentistry Ss. Cyril and Methodius University in Skopje.

After the incubation period, the plates were observed for fungal growth, using morphology and color to identify C. albicans, based on the pigmentation of the developing colonies, which is due to different enzyme activities from Candida species. This medium shows different color colonies for C. albicans (medium-sized colonies with a smooth surface, dark green to metallic green-blue) and Non-albicans Candida species (bright color, which can range from light pink, light blue to light green) (Figure.1)[16].

Figure 1. *Macroscopic appearance of the Candida species on the CHROM agar culture medium: C. albicans (metallic green-blue) Non-albicans Candida (bright color).*

Statistical Analysis

Standard methods of descriptive statistics were used. The statistical analysis was performed with the statistical program SPSS 23.0 and STATISTICA 8.0.

Results

Gender distribution showed no differences between the studied groups of patients; however, differences were observed in relation to the mean age, which were lower in the control group ($p < 0.01$).

Analyzing the presence of the plaque in examined groups (Figure 1), it was observed that dental plaque was registered in 4/20 patients in CG (18%), in 10/20 in MCP (50,8%) and in 12/20 in SCP (61,5%). PI was significantly higher in SCP compared to CG as shown in Figure 2 ($p< 0.01$).

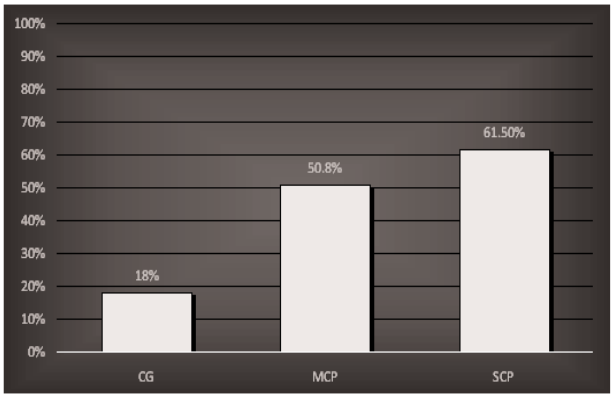

Figure 2. *The prevalence of dental plaque in examined groups: control group (CG), moderate chronic periodontitis group (MCP) and severe chronic periodontitis group (SCP).*

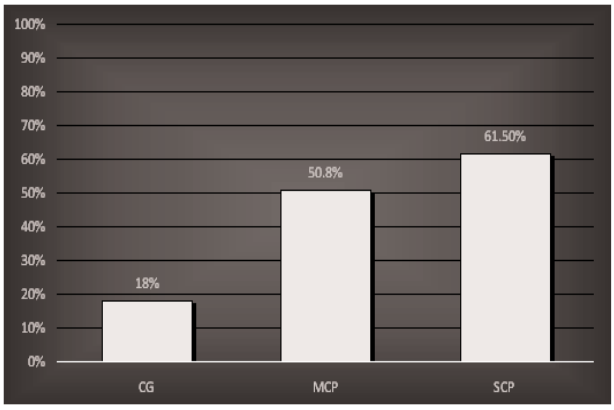

Figure 2. *Positive Candida colonization according to groups. CG-Control Group; MCP-Moderate Chronic Periodontitis group, SCP-Severe Chronic Periodontitis group.*

Considering all the individuals in each group, it was possible to observe that the most frequently colonized anatomic site was the oral mucosa, although no significant differences were found in the mucosa colonization

among the three groups ($\chi 2$, p = 0.060). Positive finding of Candida spp. at this site were found in 9/20 patients (45%) in CG, in 10/20 (49%) in MCP, and in 11/20 (52%) in SCP.

The subgingival findings of Candida spp. were positive in 3/20 (15%) at CG, in 6/20 (30%) at MCP and in 9/20 (45%) at SCP. Furthermore, a significant difference was observed in the number of total carriers in the SCP group with respect to the CG subjects (xi2; p= 0.014).

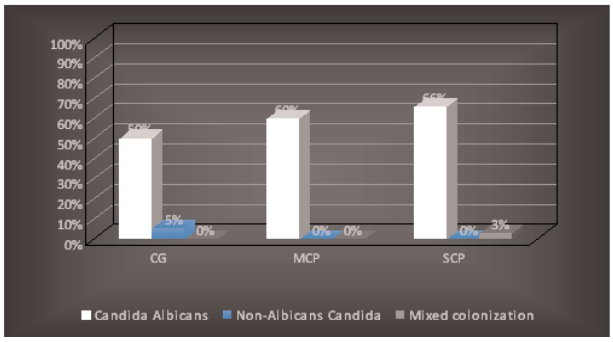

Figure 2. *Different fungal species according to the groups.*

CG-Control Group; MCP-Moderate Chronic Periodontitis group, SCP = Severe Chronic Periodontitis group.

In all studied groups, C. albicans was the most represented species. The highest percentage of C. albicans was isolated at SCP in 66% (13/20) with positive cultures, 60% (12/20) at MCP, and 50% (10/20) at CG. There were no statistical differences in the presence of C. albicans between groups (p>0.05). Non-albicans candida species were detected only at CG in 5%, they were not present in the other groups. Mixed colonization (C. albicans and Non-albicans Candida) was detected in 3% only at SCP group.

Discussion

Candida spp. colonizes the oral cavity, presenting the commensal or pathogenic properties that can be modified by direct or indirect interactions with different types of bacteria, depending on the localization of the microbial communities (e.g., supragingival plaque, subgingival plaque, and tongue coating).

Candida spp. is one of the fungi reported to be found in periodontal disease. The presence of its hyphae has been demonstrated in the connective tissue of periodontal patients in association with highly invasive anaerobic bacteria, such as Prevotella intermedia and Aggregatibacter actinomycetemcomitans[19]. These interactions, which are associated with their capacity to invade gingival connective tissue, may be important in microbial colonization that contributes to progression of oral diseases[20].

Yeasts and periodontal pathogens can interact physically, chemically, and metabolically to influence microbial survival, colonization, and biofilm formation. The pathogenic mechanism by which Candida species contribute to the progression of periodontal disease is attributed to the known virulent properties of these species, like adhesion, dimorphism, invasion, and biofilm formation, which facilitate colonization and proliferation in the oral mucosa and possibly in the periodontal pockets.

Anaerobic environment of the periodontal pocket can promote virulence of Candida spp. increasing the secretion of proteinases that damage tissues, modulate the immune response, and attract other periodontopathogens[18,21].

The subgingival biofilm, as the most complex microbial community, has been proven to be a reservoir of Candida spp. Biofilm formation is a major virulence factor in the pathogenicity of candida, and candida biofilms are difficult to eradicate especially because of their very high antifungal resistance[22].

A strong positive and significant correlation between Minimal Inhibitory Concentration MICs for subgingival isolates and full-mouth plaque score (FMPS) was also obtained, as a marker of oral hygiene for all antimycotics. This means that with a lower level of oral hygiene, which may provide conditions for maturity and higher complexity of biofilms, the MIC values increase. The susceptibility of C. albicans to antifungals correlates with oral hygiene and the severity of periodontal destruction[23].

In the present study (Figure1), dental plaque was detected in all studied groups, the expected lowest percentage (15%) was registered in the gingival sulcus in the control group, and the highest in the periodontal pockets in SCP (61.5%).

According to the obtained results from the present study, candida was present both in the oral mucosa and in the subgingival parts, in the studied subjects (Figure 2). The oral mucosa is still a place where candida develops in a higher percentage 45%, 49% and 52% in CG, MCP and SCP respectively, compared to the subgingival region (15%, 30% and 45%). The only exception is SCP, where an approximate percentage of candida (45%) was also registered in the subgingival region.

Several studies[25,26] have indicated a 35% prevalence of yeasts in oral mucosa in periodontally healthy subjects, which is somewhat lower than the 45% observed in this study. With respect to the subgingival sites of the CG, colonization of 15% suggest that, under normal conditions, yeast does not develop easily in the subgingival sites (Figure 2). The results from the present study are in accordance with previous studies (27,28,29) claiming

that not all mucosa carrier patients were also carriers in the subgingival sites, suggesting that the entrance of the yeasts to the subgingival areas is restricted. According to the study by Radunovic et al.[23], the prevalence of Candida spp. in subgingival areas in healthy subjects was up to 70%. Further, the cohorts of healthy subjects with periodontitis showed lower frequency of Candida spp. on the tongue, but the presence of subgingival Candida spp. varied from 14.3% to even 26.7%[30]. These studies initiate that subgingival areas may differ in incidence and/or species distribution of Candida from the oral mucosa, because of their differences. Subgingival biofilm is attached to a non-shedding hard surface, with different primary colonizers, pH and electrochemical potential and nutrients in the subgingival plaque differing from the tongue as well as the availability of oxygen, giving the subgingival area the potential to develop different biofilms from the tongue[31].

Most of the published studies about association of yeasts and periodontitis do not provide precise information relative to the severity of the disease. Several studies have indicated lower prevalence of yeasts in subgingival sites (17%), unlike 52,5% observed in the total sample of periodontitis patients in this study[8,11,13,17].

The percentage of yeast carriers in subgingival sites in periodontitis groups were similar. On the other hand, although no significant differences were found in the subgingival colonization among MCP (30%) and SCP (45%) (Figure 3), patients with SCP had a greater percentage of colonization than MCP and CG (15%).

Jabri et all.[32], reports that the prevalence of subjects with yeasts in the examined periodontal pockets has been ranging from 7.1- 9.6% to 15.6%. This suggests that subgingival colonization by candida species could be favored in chronic periodontal disease, and that they have a role to play in the infrastructure of periodontal microbiota[33]. The meta-analysis results demonstrated that Candida spp. detection rate and density were statistically significantly higher in CP patients than in subjects with clinically healthy periodontium. Whether this fact is the cause or consequence of periodontal disease, remains unclear[17].

As already mentioned, Candida colonization of the oral mucosa is not always associated with its presence in the subgingival regions, especially in patients who do not have periodontal pockets, but in already formed pockets with ulcerated and degenerated epithelium, there is probably a greater possibility of Candida entering the subgingival regions from the oral mucosa. It has been suggested also, that its presence in the subgingival area could be transient[34].

According to the obtained percentages for the subgingival presence of candida in the present study (CG-15%, MSC-30% and SC-45%), subgingival area as a reservoir of yeasts should be seriously considered. Candida spp. in subgingival areas is more resistant because it is always present within biofilms, and additionally these sites are inaccessible to conventional antifungal drugs[35]. In addition, refractory periodontitis, resistant to conventional therapy and requires systemic antibiotic therapy, may be associated with uncontrolled fungal growth in the periodontal pocket[36].

Usually, in clinical praxis, when candidiasis is suspected to be present, oral swabs are taken only from the oral mucosa, but not from the subgingival area. The mucosal colonization by C. albicans did not always ensure its presence in the periodontal pockets (confirmed by the lower percentage of candida in the subgingival parts vs mucosa)[37]. In contrast to this study, some cases were documented where C. albicans was isolated from periodontal pockets but not from the mucosa. This indicates that, in order to see the global picture of the yeast microbiota in the oral cavity, it is necessary to sample both the mucosa and the periodontal pockets[23,38].

With respect to the prevalence and yeast species profile, in mucous and subgingival sites, three different variants were identified at examined subjects: Candida albicans, Non-albicans Candida infection and mixed colonization (Figure 4). Candida albicans was the dominant yeast species found in both anatomical sites in all three groups. Although several yeast species were found, only C. albicans was present in all yeast-positive patients.

In the present study, ten periodontal healthy patients (50%) harbored C. albicans in the subgingival plaque. These results are high in comparison to other studies (8,36) which showed variable occurrence (16% to 36%) of C. albicans in the subgingival plaque of the healthy periodontium. Twelve patients (60%) with MCP presented yeasts in the subgingival biofilm, while thirteen patients (66%) in the SCP group were positive for these microorganisms. No statistical difference was observed between the examined groups ($P = 0.084$).

In the cases of patients without periodontitis (CG), only 5% of Non-albicans species were noted. The presence of Non-albicans species were not detected in MCH and SCH groups, except mixed colonization present only in SCP (3%). The results reported in this work, indicate that there are no significant differences in the characteristics of the yeast microbiota recovered from patients with CP when compared to patients without periodontitis. Also, the stages of the disease does not affect the type of candida. A possible explanation could be that the microenvironment hinders the co-existence of C. albicans with other yeast species, or where only C. albicans is capable of surviving given its wide range of virulence factors[39].

Urzúa's study[27] suggests that the degree of colonization is not related to the depth of the periodontal pocket in aggressive (AP) and chronic periodontitis (CP) groups, but with differences in the profile and in the diversity of species. The subjects with AP had C. albicans at all three depths, while C. dubliniensis, C. glabrata and C. albicans were noted in the CP patient population, probably related to co-existing of the periodontopathogens. In a study carried out by Popova et al.[40], no Candida species were observed in patients with chronic periodontitis, showing a negative correlation.

Certain studies report that detection rate of species such as C. glabrata, C. krusei, C. tropicalis and C. parapsilosis was similar in periodontal pocket samples and crevicular samples in CP patients and subjects with clinically healthy periodontium, respectively[41,42]. Colonization of periodontal pockets by these Non-albicans specie did not necessarily certify their activity in the pathogenesis of periodontitis. They can be transient members of the microbial consortium and can be evaluated as a potential reservoir for systemic distribution in case of favorable conditions[17].

Conclusions

Although the role of C. albicans in CP has not yet been established, this yeast is considered an important pathogen implicated in the pathogenesis of the tissue-destructive periodontal disease. However, further researches are needed to clarify the exact pathogenic mechanism of this opportunistic fungus in periodontal diseases.

Acknowledgements

We would like to express our gratitude to the Faculty of Dentistry, Ss. Cyril and Methodius University in Skopje, N. Macedonia, for the opportunity to conduct a microbiological research in the biochemical laboratory, providing the equipment necessary for conducting the study.

Declaration of Interest

We hereby state that no financial or material support was received for the work. No affiliation or organization will be affected by the material in the manuscript. We state that there is no conflict of interest.

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