



COLONIZATION OF *CANDIDA* SPP. IN PATIENTS TREATED WITH PARTIAL DENTURES “THERMOSENS”

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ABSTRACT

Introduction. One of the commonly encountered pathologies in the oral cavity when using removable dentures is denture stomatitis, attributed to *Candida* spp. colonization on the denture surface.

Aim. Determination of the type and quantity of *Candida* spp. on the mucosal and denture surfaces in patients treated with “ThermoSens” partial dentures before and after treatment.

Materials and Methods. Thirty patients were prosthetically treated and divided into 2 groups. In the first group (n=20), treatment was performed with “ThermoSens” partial dentures, while in the second (control) group, patients were treated with conventional dentures (n=10). Swabs were taken from the mucosal and denture surfaces. The samples were tested for quantitative and qualitative differentiation of *Candida* spp. before prosthetic treatment, at the first and third month after prosthetic treatment. The data was statistically processed using SPSS Statistics 26.

Results. No statistically significant difference was observed regarding the quantitative accumulation of *Candida* spp. on the denture and mucosal surfaces between the two groups of patients ($p>5\%$), measured at 1 month and 3 months after prosthetic treatment. It was found that the predominant *Candida* spp. in both groups of patients was *Candida albicans*.

Conclusion. Although no statistically significant difference was observed in the two test groups regarding the number of colonies of *Candida* spp., the main group showed a tendency for a more significant increase in their number (up to 10^5 CFU/ml) on the denture surfaces at the third month after prosthetic treatment, which may be a pre-sumption for the development of denture stomatitis.

Keywords: flexible dentures, “ThermoSens”, *Candida* spp.,

INTRODUCTION

Polymethylmethacrylate (PMMA) is the most versatile denture base material in contemporary removable prosthodontics due to its acceptable aesthetic quality and satisfactory mechanical and physical properties. Other than their numerous advantages, acrylic denture base resins have several disadvantages, such as dimensional inaccuracy, brittleness and allergy to methyl methacrylate monomers [1, 2]. The constant improvement in patients’ quality of life and their increased requirements regarding comfort, durability, esthetics, and biocompatibility have led to an increase in the use of thermoplastic polyamide dentures in daily practice.

These dentures are hypoallergenic [3], making them ideal for use in patients with known allergies or other sensitivities [4]. They also offer increased comfort, durability and esthetics compared to conventional dentures [5]. Despite their advantages over conventional dentures, thermoplastic polyamide dentures have shown some shortcomings, as reported by several authors [6, 7]

The primary disadvantage of thermoplastic polyamides is determined by the surface characteristics of the material, specifically easier surface damage, as well as matting and loss of colour [8]. Although it is possible to achieve the same level of polishing and fineness as conventional dentures [9], when it comes to the surface roughness (RA) of thermoplastic polyamide dentures, mistakes during the manufacturing process and polishing in the dental office [10] can lead to increase of the surface roughness of the denture and an increase in the retention of *Candida* spp [11, 12]. The oral cavity is home to many different species of *Candida*. The most commonly observed are *C. albicans* and *C. glabrata* [13]. This, combined with other factors, such as the patient’s standard of oral hygiene and health condition, the humidity and temperature present in the oral cavity, are factors for increased colonization of the removable dentures [14]. Studies have shown that there is a gradual increase in the number of *Candida* spp. colonies in patients wearing prosthetics, even when a strict disinfection protocol is followed [15, 16].

The most commonly observed disease of the oral mucosa is denture-related stomatitis (DRS); it is present in

the majority of denture wearing patients and is often asymptomatic, though extreme cases can be observed [15, 17]. Three different types of DRS can be observed according to Newton's 1962 study. Although *Candida albicans* is considered to be the main cause of DRS, more recent studies have shown a synergistic interaction between *C. albicans* and *C. glabrata* which increases the colonization capability of these microorganisms to the denture surface [13].

The quantity of *Candida* spp. isolated from swabs taken from the denture surface, the underlying oral mucosa can vary, and there is no consensus on the amount of colony-forming units per milliliter (CFU/mL) that lead to symptomatic DRS [18].

The aim of the present in vivo study is to determine the type and quantity of *Candida* spp. on the mucosal and denture surfaces in patients treated with "ThermoSens" partial dentures before and after treatment.

The null hypotheses of the present study were: **1.** There is no difference in colonization of *Candida* spp. between patients treated with "ThermoSens" dentures and patients from the control group (patients treated with conventional dentures). **2.** There is no quantitative change in colonization of *Candida* spp. over time in patients treated with "ThermoSens" dentures.

MATERIALS AND METHODS

In the Faculty of Dental Medicine – Sofia, 30 patients above the age of 18 were treated with removable partial dentures and were distributed in two groups according to the material their dentures were made of.

- First group (Group A) – patients were treated using thermoplastic polyamide partial dentures "ThermoSens" by "Vertex Dental" in either the upper or lower jaw.

- Second group (Group B, control group) – patients were treated using conventional polymethylmethacrylate (PMMA) partial dentures in either the upper or the lower jaw.

All patients signed an informed consent form to participate in the present research. The scientific research was approved by the Research Ethics Commission 'KENIMUS' (statement No. 10/03.07.23).

Before prosthetic treatment, samples were collected from the mucosal surfaces (buccal mucosa and tongue) of patients from both groups (n=30) for microbiological examination to exclude elevated levels of *Candida* spp.

In the first and third month following prosthetic treatment, samples were collected from the mucosal surfaces (buccal mucosa and tongue) and from the internal surface of the prostheses for species-specific and quantitative determination of *Candida* spp. for patients from both groups.

To standardize the hygiene factor of the prosthetic constructions, all patients included in this study were provided with the same denture cleaning tablets (Protefix, Germany) for the entire period (3 months).

Selection of patients – Exclusion criteria

Patients with systemic diseases (such as asthma, diabetes mellitus, Sjögren's syndrome, immunodeficiency con-

ditions), patients who have taken antibiotics in the last 3 months, patients undergoing radiation or chemotherapy in the last 6 months, those diagnosed with denture stomatitis as a result of wearing old plaque-retained dentures, and those with gingival and periodontal diseases were excluded from the study.

Microbiological Examination

Sterile swabs with AMIES transport medium 300290 (Ecomet-90 Ltd.) were used to collect swabs from the buccal mucosa, tongue and denture surface. The sterile swabs were carefully removed from their packaging and used to gently swab the surface of the buccal mucosa, tongue and denture surface. Subsequently, the swabs were placed in the nutrient medium provided with the kit and transported to the microbiology laboratory in a cooler bag at a temperature of 4°C.

The presence and quantity of *Candida* spp. (*C. albicans*, *C. glabrata*, *C. tropicalis*, etc.) on mucosal surfaces will be determined by:

- Culturing the clinical material on solid and liquid nutrient environment to demonstrate the fungal agent.
- Utilizing fermentation and assimilation tests for the identification of different species of *Candida* spp.

Statistical methods

The statistical methods used are as follows:

- Descriptive statistics - absolute (N) and relative (%) values - used to determine the number of patients and their percentage expression.

- Non-parametric test for two independent samples (Mann-Whitney Test) - the choice of the test is dictated by the fact that the quantity of *Candida* spp. is on an ordinal scale and differences are sought between patients from two groups: Group A and Group B.

- Non-parametric test for two related samples (Wilcoxon Test) - the choice of the test is dictated by the fact that the quantity of *Candida* spp. is on an ordinal scale, and differences are sought between two periods on the prosthetic surface: 1-month post-prosthesis placement and 3 months post-prosthesis placement.

- Non-parametric test for three independent samples (Friedman Test) - the choice of the test is dictated by the fact that the quantity of *Candida* spp. is on an ordinal scale, and differences are sought between three periods on the mucosal surface and denture surface: before prosthesis placement, 1 month post-prosthesis placement, and 3 months post-prosthesis placement.

The statistical methods were applied using IBM SPSS Statistics 26, and graphical representation was carried out using Excel 2013. All tests were conducted at a 5% risk of error.

RESULTS

The quantity of *Candida* spp. was measured in colony-forming units per milliliter (CFU/mL) and was conditionally divided into the following groups:

- Norm: $<10^3$ CFU/mL.
- Light: $\geq 10^3 < 10^4$ CFU/mL.
- Moderate: $\geq 10^4 < 10^5$ CFU/mL.
- Heavy: $\geq 10^5$ CFU/mL.

Comparing the quantity of *Candida* spp. on the oral mucosa - the comparison at the mucosal surface was conducted separately between patients from Groups A and B before denture delivery, 1 month and 3 months post-denture delivery.

The analysis indicates that there was no statistically significant difference in the quantity of *Candida* spp. between patients from Group A and Group B at the mucosal surface at any of the tested time points, as indicated in Table 1.

Table 1. Results of the examination for the difference in the quantity of *Candida* spp. between patients from Group A and Group B.

Time	Groups	Quantity <i>Candida</i> spp. [CFU/mL]	Comparison		Man-Whitney Test
			Group A	Group B	
Before denture delivery		Norm	80%	50%	p > 0.05
		Light	15%	40%	
		Moderate	-	10%	
		Heavy	5%	-	
Total			100 %	100 %	
1 month post-denture delivery		Norm	40%	30%	p > 0.05
		Light	35%	50%	
		Moderate	25%	20%	
		Heavy	-	-	
Total			100 %	100 %	
3 months post-denture delivery		Norm	20%	10%	p > 0.05
		Light	40%	70%	
		Moderate	25%	20%	
		Heavy	15%	-	
Total			100 %	100 %	

Comparing the quantity of *Candida* spp. on the denture surface - the comparison at the denture surface was conducted separately between patients from Group A and B, 1 month and 3 months post-denture delivery.

The analysis indicated that there was no statistically significant difference in the quantity of *Candida* spp. between patients from Group A and Group B at the prosthetic surface at any of the tested time points, as presented in Table 2.

The analysis indicated that there was no statisti-

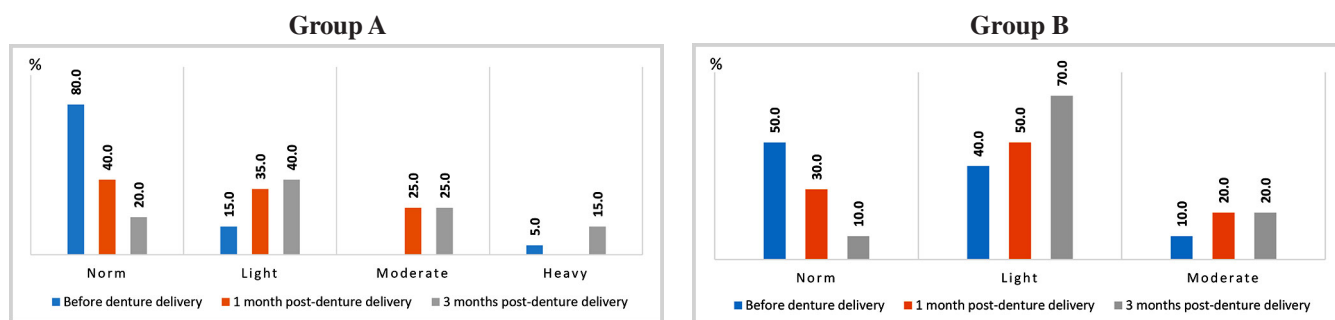
Table 2. Results of the examination for the difference in the quantity of *Candida* spp. isolated from the denture surface between patients from Group A and Group B

Time	Groups	Quantity <i>Candida</i> spp. [CFU/mL]	Comparison		Man-Whitney Test
			Group A	Group B	
1 month post-denture delivery		Norm	45%	40%	p > 0.05
		Light	30%	40%	
		Moderate	10%	20%	
		Heavy	15%	-	
Total		100 %	100 %		
3 months post-denture delivery		Norm	10%	30%	p > 0.05
		Light	45%	50%	
		Moderate	20%	20%	
		Heavy	25%	-	
Total		100 %	100 %		

At the mucosal surface - the comparison at the mucosal surface was conducted separately between the quantities of *Candida* spp. before denture delivery, 1 month and

3 months post-denture delivery for patients from Group A and Group B.

Fig. 1. Percentage distribution of the quantity of *Candida* spp. in patients from a) Group A and b) Group B before denture delivery, 1 month and 3 months post-denture delivery ($p < 0.05$).

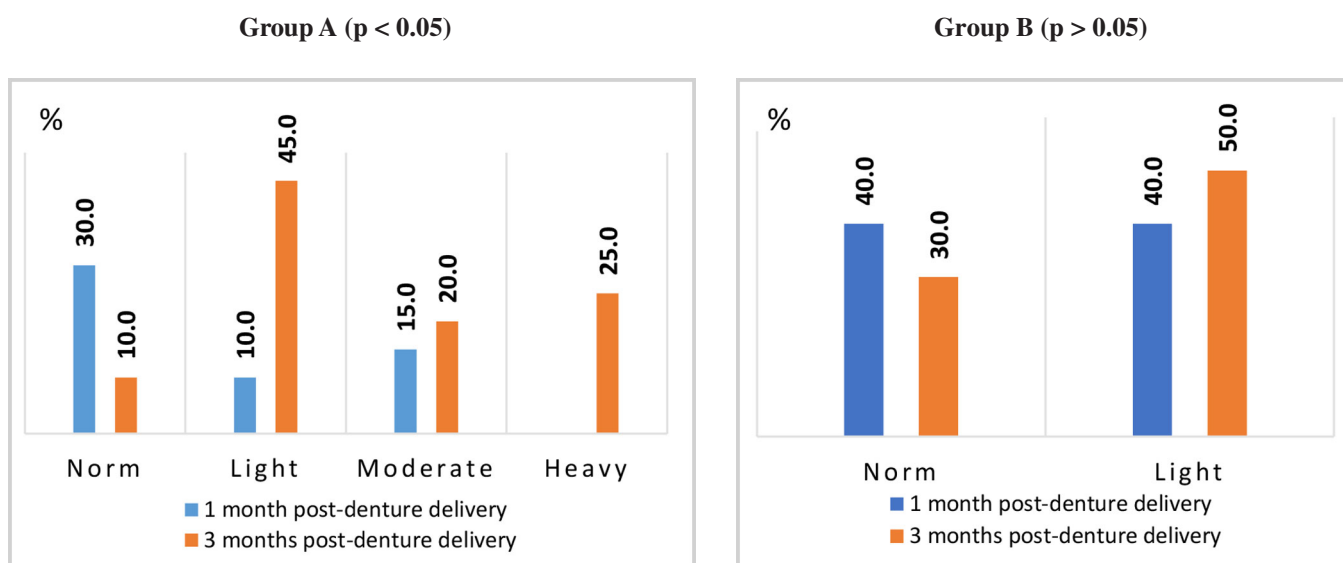


In Group A, it was observed that the percentage of detected quantities of *Candida* spp. migrated towards higher percentages 3 months post-denture placement compared to before denture placement. At the end of the period, mass quantities of *Candida* spp. were detected in this group in 15% of patients. In Group B, a similar migration was observed, but it manifests as a significant increase in the percentage of patients with mild quantities of *Candida*

spp. (70%). The examination shows that both in patients from Group A and Group B, there was a substantial increase in *Candida* spp. levels over time. This is demonstrated in Figure 1.

At the denture surface - the comparison at the prosthetic surface was conducted separately between the quantities of *Candida* spp. 1 month and 3 months post-denture delivery for patients from Group A and Group B.

Fig. 2. Percentage distribution of the quantity of *Candida* spp. isolated from the denture surface in patients from a) Group A and b) Group B 1 month and 3 months post-denture delivery.



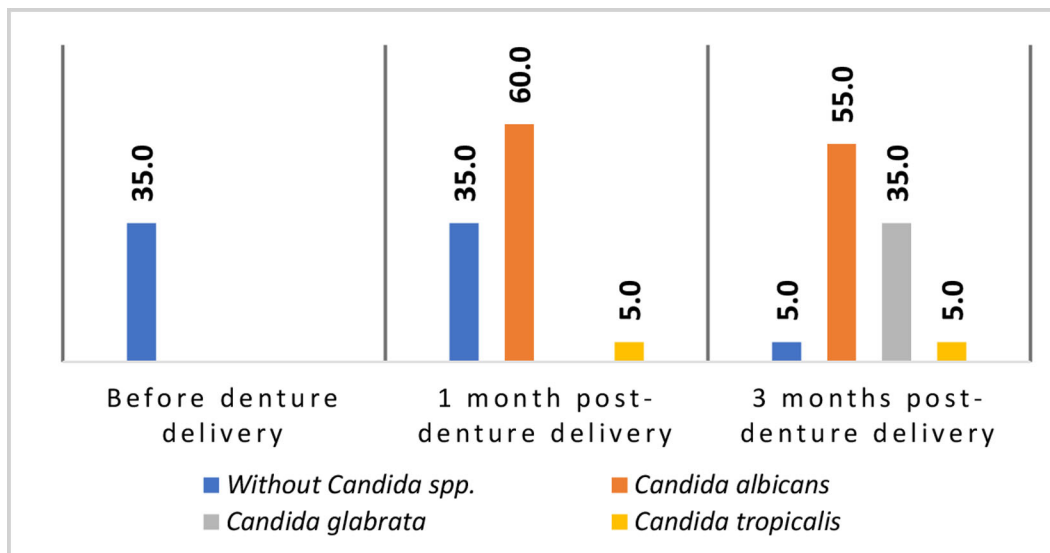
In Group A, a noticeable migration is observed in the percentage of patients from low to high levels of *Candida* spp., with 25% of patients exhibiting “heavy” quantities 3 months post-denture placement. In Group B, such clear migration was not observed. The examination shows that in patients from Group A, there was a substantial increase in *Candida* spp. levels over time. However, such an increase was not observed in Group B, Figure 2.

In addition to the quantitative differentiation of *Candida* spp. on both the mucosal and denture surfaces

over the course of the current study, we also performed qualitative differentiation regarding the most predominant type of *Candida* spp. isolated from each surface.

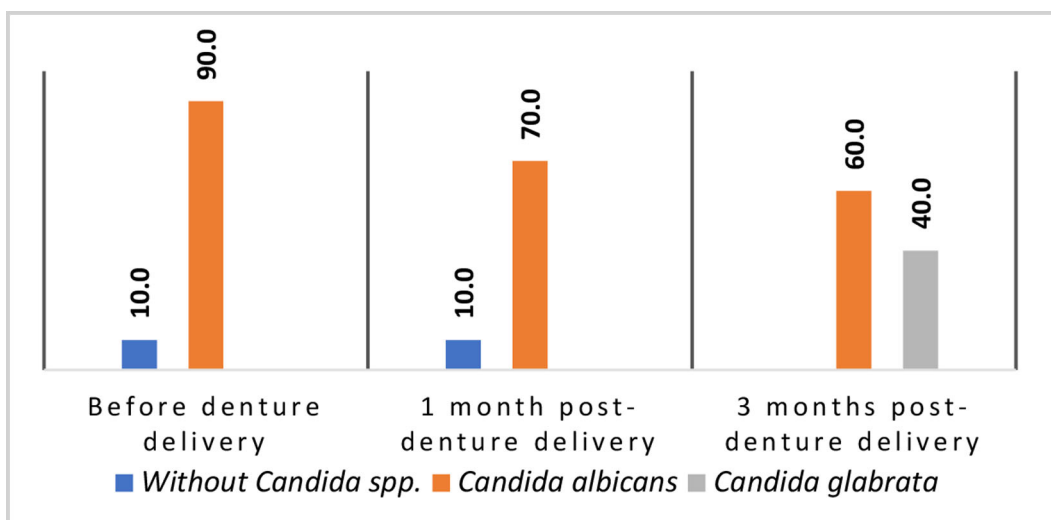
The predominant species of *Candida* spp. among patients from Group A is *Candida albicans*. It was detected in 60% of patients 1 month post-denture placement and in 55% of patients 3 months post-denture placement. The next most prevalent species of *Candida* spp. is *Candida glabrata*, with *Candida tropicalis* being the least prevalent, as presented in Figure 3.

Fig. 3. Percentage distribution of *Candida* spp. species over time among patients from Group A.



Among patients from Group B, only two species of *Candida* spp. were detected: *C. albicans* and *C. glabrata*. It can be noted that *C. albicans* dominates, being present in 90% of patients before denture placement, in 70% of patients 1 month post-denture placement, and in 50% of patients 3 months post-denture placement, as presented in Figure 4.

Fig. 4. Percentage distribution of *Candida* spp. species over time among patients from Group B.



DISCUSSION

The collection of swabs from the oral mucosa and the inside denture surface remains one of the most accurate methods to determine the amount and type of *Candida* spp. in the oral cavity [19]. The lack of publications regarding *Candida* spp. colonization in patients treated with thermoplastic polyamide dentures is one of the reasons we conducted this comparative study.

The results we obtained regarding the quantity of *Candida* spp. isolated from the denture surface in patients treated with flexible partial denture “ThermoSens” when compared to patients treated with conventional PMMA dentures reject our initial hypothesis that *Candida* spp. would accumulate more on the surface of the polyamide dentures. Different authors have accredited the increase in surface roughness of the dentures of the different types of

denture cleansers that patients use, which can lead to higher *Candida* retention [8]. Our analysis of the results shows no statistically significant difference could be found in the three observed periods of time between the two groups (Group A and Group B) of investigation. Our findings are similar to those made by Olms C, et al. [20]; however, they contradict the findings by de Freitas Fernandes FS, et al [21], which concluded that the polyamide material promoted more biofilm growth as compared to the PMMA.

Again, when comparing the quantity of *Candida* spp. colonization of the oral mucosa in Group A and Group B, we do not establish a statistically significant difference between the two groups at any of the three tested periods. A study conducted in 2019 by Ahmed E, et al. [22] came to the conclusion that flexible nylon-based dentures showed lesser *Candida* adherence on the denture fitting area than the con-

ventional heat-cured acrylic resin, another study carried out by Sampaio-Fernandes M, et al. [23] states the opposite.

Comparing the quantities of *Candida spp.* on both the oral mucosa and the denture surface at different time points to determine if they change over time, taking into account the patient group (Group A and Group B) has shown that although the colonization on the oral mucosa increased at a similar rate in both groups, when it comes to the denture surface a significant increase of colonies was observed in Group A. This can be contributed to the different surface characteristics of the polyamide denture base or wrong polishing protocols in the dental office [6], as well as the continual use of dental cleansers that continuously increase surface roughness over time [8], even though both types of denture base materials have similar RA values using the same polishing techniques [9].

A study carried out in 2021 by Devcic et al.[24] concluded that although the prevalent species of candida isolated from conventional PMMA dentures is *C. albicans*, over time, the amount of *C. glabrata* increases, which is in accordance with the results obtained in the present study. Some authors suggest that there is a synergistic reaction between *Candida albicans* and *C. glabrata*, which may contribute to increased adhesion to the denture surface [25].

A study carried out by Badaró MM, et al. [16] shows that accumulation of a biofilm on the removable denture occurs even in patients who follow an extensive disinfection protocol. The formation of such a *Candida* biofilm ultimately results in the failure of the removable denture and the subsequent development of DRS [26].

CONCLUSION

Although some percentage differences were observed, the analysis shows that there is no statistically significant difference between the quantity of *Candida spp.* on both the oral mucosa and the denture surface. This provides grounds to assert that the quantity of *Candida spp.* between patients from Group A and Group B does not differ significantly. The analysis of the data to determine whether there is a significant change in the quantity of *Candida spp.* overtime on the oral mucosa and on the denture surface shows a difference for Group A across all surfaces: mucosal surface and denture surface. For Group B, a difference over time was only observed at the mucosal surface.

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