

Prevalence of *Candida* spp. during radiographic examination in Diabetes mellitus patients

Prevalência de Candida spp. durante o exame radiográfico em pacientes diabéticos

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Resumo

Introdução: Relata-se que indivíduos diabéticos são mais susceptíveis a infecções por *Candida* que indivíduos saudáveis, especialmente se doença periodontal estiver associada. **Objetivo:** Este estudo propôs avaliar a prevalência de colonização por *Candida* spp. durante o exame radiográfico em pacientes diabéticos e não diabéticos. **Material e método:** Vinte e seis pacientes com Diabetes mellitus do tipo 2 e 20 pacientes sem Diabetes mellitus, apresentando periodontite crônica e *Candida* spp. na saliva, foram avaliados. Durante o exame radiográfico, amostras de saliva foram coletadas: da mucosa oral, do filme radiográfico periapical convencional, sensor radiográfico digital (CDR) e bloco de mordida do posicionador de filmes. Unidades formadoras de colônia (cfu/mL) e identificação das leveduras do gênero *Candida* foram avaliadas. **Resultado:** A mucosa oral de ambos os grupos mostrou maior colonização por *Candida* spp. quando comparada com outras superfícies coletadas ($p < 0.05$). Nos pacientes diabéticos, a mucosa da região esquerda superior mostrou níveis mais altos de colonização. Nos pacientes não diabéticos, a região de molar superior direito mostrou o nível mais alto de colonização durante o exame no posicionador, no sensor e no lado do filme periapical que não fica voltado para a radiação X. Os níveis de *Candida* spp. na saliva foram similares entre diabéticos (média = 3.0×10^6) e não diabéticos (média = 3.8×10^6). **Conclusão:** Nenhuma diferença na colonização por *Candida* spp. (cfu/mL) em pacientes diabéticos e não diabéticos foi observada nas cinco superfícies coletadas e nas regiões radiográficas simuladas. *Candida albicans* foi a espécie prevalente de *Candida* spp. encontrada em todas as amostras.

Descritores: Doenças periodontais; diabetes mellitus; radiografia dentária digital.

Abstract

Introduction: It is suggested that individuals with diabetes are more susceptible to *Candida* infections than healthy people, especially if periodontal infection is associated. **Objective:** This study evaluated the prevalence of colonization by *Candida* spp. during radiographic examination in diabetic and non-diabetic patients. **Material and methods:** Twenty-six patients with type 2 diabetes mellitus and 20 patients without diabetes mellitus, presenting chronic periodontitis and presence of *Candida* spp. in saliva were evaluated. During radiographic examination, samples of saliva were collected from: oral mucosa, conventional radiographic periapical film, digital x-ray sensor (CDR), and bite block of the receptor-positioning device. Colony forming units (cfu/mL) and identification of *Candida* yeasts were assessed. **Result:** Oral mucosa from both groups showed the highest colonization with *Candida* spp. if compared with others surfaces collected ($p < 0.05$). In diabetic patients, the mucosa of the upper left regions showed higher levels of colonization. In non-diabetic patients, the upper right molar region showed the highest level of colonization during the examination of the receptor-positioning device, the sensor and the non-sensitive film. *Candida* spp. levels in saliva were similar between diabetics (mean = 3.0×10^6) and non-diabetics (mean = 3.8×10^6). **Conclusion:** No difference in *Candida* spp. colonization (cfu/mL) in diabetics and non-diabetic patients was observed for the five collected surfaces and the simulated radiographic region. *Candida albicans* was the prevalent species of *Candida* spp. found on all the samples.

Descriptors: Periodontal diseases; diabetes mellitus; dental digital radiography.

INTRODUCTION

Infection-control practices are designed to create and maintain a safe clinical environment to eliminate or minimize disease transmission during patient treatment¹. There is high potential for cross-contamination of equipment and environmental surfaces with blood or saliva when taking dental radiographs². Saliva has always been considered a potentially infectious material in dental infection control². White, Glaze³ found that dental healthcare workers can transfer oral microorganisms from the patient's oral cavity to radiographic equipment during routine intraoral radiography. These microorganisms remain viable on radiographic equipment for at least 48 hours.

Traditionally, intraoral radiographs are acquired using films. Since the introduction of digital radiography to dentistry, many dental schools and private practices have adopted digital imaging methods for acquiring radiographs. Digital radiology promises many advantages over traditional film-based techniques^{4,5}. Digital radiography sensors come into contact with mucous membranes and are considered semicritical devices. They should be cleaned and ideally heat-sterilized or high-level disinfected between patients. However, there is a variety in the ability of digital radiographic sensors to be sterilized or high-level disinfected². Semicritical items that cannot be reprocessed by heat sterilization or high-level disinfection should, at a minimum, be barrier protected by a plastic barrier sheaths to reduce gross contamination during use that is not guaranteed from contamination^{2,6}.

Previous reports mentioned that individuals with diabetes present oral complications more frequently than healthy people⁷. Commonly, these oral complications are associated with fungi, for instance, *Candida* spp., which presents as white or white-yellow color, creamy, convex colonies, with a smooth and bright appearance, that are moist with a typical smell⁸. These oral complications are also associated with *Candida* species that have frequently been isolated from the oral cavities of patients with diabetes mellitus⁹⁻¹⁵. Besides, evidence indicates a strong correlation between the severity of periodontitis and diabetes, based on the fact that periodontal infection is associated with poor glycemic control in this particular group of patients^{7,16,17}. Furthermore, it has been established that the higher occurrence rate of yeasts is associated with immunocompromised patients with periodontal diseases, such as patients with diabetes^{12,13,18}.

Moreover, patients with long-standing, poorly controlled diabetes are at risk of developing oral candidiasis¹⁹. Although authors had related that 40% of the patients colonized with candidal species had no clinical signs of oral candidosis²⁰. A number of candidal species were recovered from the oral cavity of insulin-treated diabetic patients. *C. albicans* was the most commonly recovered species, recovered from 85% of the diabetic patients. *C. dubliniensis* was the second most commonly occurring species²⁰.

Considering the lack of data about the prevalence of colonization by *Candida* spp. in diabetic and non-diabetic patients

with periodontal disease during radiographic examination, this study was carried out in order to determine what surfaces would be contaminated. Therefore, the aim of this study was to determine the prevalence of colonization by *Candida* spp. in diabetic and non-diabetic patients during radiographic examination, on different surfaces.

MATERIAL AND METHODS

1. Sample

This study was approved by the Ethics in Human Research Committee of the Dental School (protocol number 79/03). All volunteers were informed about the aims and methods of this study, and gave their written consent to participate. The sample size calculation was based on previous studies²¹⁻²³. A post-hoc statistical power calculation test was performed based on cfu/mL for both groups and all evaluated surfaces, and the sample size was estimated in 20 patients per group, considering a power of 80%.

As inclusion criteria, all subjects that participated in this study must have been presenting chronic periodontitis and presenting *Candida* spp. in their saliva, regardless of gender, race, social group, age, oral hygiene, and nutrition habits. The following exclusion criteria were considered: history of antibiotic therapy within the previous 6 months and anti-inflammatory drugs within the previous 3 months; current history of immunosuppression; local or systemic use of antifungal drugs and use of mouthrinses.

2. Periodontal Analyses

Chronic periodontitis was established as probing pocket depth (PPD) and clinic attachment loss (CAL) ≥ 4 mm and bleeding on probing in more than three sites in non-adjacent teeth²⁴.

3. Diabetes Analyses

The glycated hemoglobin exam (HbA1c; A1c) was requested for diabetic patients. The patient was classified as having adequate metabolic control when presenting results from the glycated hemoglobin test A1c $< 7\%$ ²⁵.

4. Saliva Analyses

The saliva collection was performed without stimulation, by asking the patients to retain their saliva and then deposit it in a funnel and bottles previously identified and sterilized, until obtaining 3 mL of saliva for microbiological analysis. The tubes containing saliva were dispersed by vortexing for 60 seconds (Vortex, Marconi Equipamentos para Laboratório Ltda., Piracicaba, SP, Brazil) and the saliva was diluted in a decimal series of 10^{-1} to 10^{-4} in phosphate-buffered saline. The saliva was then inoculated in Petri dishes with SDA and incubated at 37 °C for 48 hours, for *posteriori* microbiological analyses of presence and quantity (CFU/mL) of *Candida* spp.

Out of a total 153 periodontitis patients evaluated, 26 patients with diabetes mellitus and 20 patients without diabetes mellitus that presenting *Candida* spp. in their saliva were included in this study.

5. Radiographic Examination

For all participants who required full-mouth radiographic examination, a set of 14 radiographs was used. When the patient already had a radiographic examination, a simulation of the examination was performed with written consent from the participant. Then, 26 diabetic and 20 non-diabetic patients were submitted to radiographic simulation/examination.

Before using the conventional periapical radiographic film (Kodak Insight, Eastman Kodak Company, Rochester, NY, USA) this material was placed in a plastic barrier that was sealed (Odontobrás, Ribeirão Preto, SP, Brazil) for 4s. The CDR sensor (Schick Technologies, Long Island City, NY, USA) was also protected with a plastic barrier. The conventional film and the CDR sensor were then disinfected by rubbing with sterile gauze dipped in 70% alcohol.

The intraoral radiographic examination (or the simulation) using conventional periapical radiographic film was taken with a Rinn film holder (XCP Instruments, Elgin, IL, USA). Initially radiographs were taken with conventional film from the regions solicited, after that, the CDR sensor was maintained in the mouth for 30 seconds, with the patient's fingers, simulating the technique on five randomized regions in all patients. The regions were: upper right molar (Urm), upper right bicuspid (Urb), upper right cuspid and lateral incisor (Urc), upper central incisor (Uci), upper left cuspid and lateral incisor (Ulc), upper left bicuspid (Ulb), upper left molar (Ulm), lower left molar (Llm), lower left bicuspid (Llb), lower left cuspid and lateral incisor (Llc), lower central incisor (Lci), lower right cuspid and lateral incisor (Lrc), lower right bicuspid (Lrb), and lower right molar (Lrm).

6. Microbiological Examination

Before the radiographic examination, saliva was collected from the oral mucosa of each patient with a sterile swab that was humidified in sterile phosphate buffered saline (PBS) solution. Each swab was then rubbed onto a culture plate of Sabouraud Dextrose Agar (SDA, Acumedia, Neogen, Lansing, MI, USA)²⁶ for *posteriori* microbiological analyses.

After the radiographic examination or simulation in each region, the conventional radiographic periapical film and the randomized regions of the sensor, still with the plastic barrier, were pressed into the SDA plate using sterile clinic tweezers; this procedure was carried out on both sides of the radiographic film and only on one side of the sensor. Only one side of the sensor is submitted to X-ray which was used because the other side presents a wire connection to the computer and this side would damage the procedure of pressure of the sensor into the DAS plate. Material was also collected from the surfaces of the RINN device, which remained in contact with the radiographic film, using a sterile swab, which was then maintained in sterilized

saline solution that was then rubbed onto the culture medium on the plate. Samples were incubated at 37 °C for 48 hours, for *posteriori* microbiological analyses of quantity (cfu/mL) and identification of *Candida* species.

7. Identification of *Candida* spp.

After colony growth, the counting of the inoculum was performed in colony forming units *per* mL (cfu/mL). The characteristic colony morphology of the *Candida* yeasts was demonstrated using a stereoscopic microscope (Carl Zeiss do Brasil Ltda, Brazil) and different colonies were identified by Gram staining. A test in CHROMagar® *Candida* chromogenic medium (Difco, BD) was performed to identify the *Candida* species^{27,28} and other identifying tests were also carried out according to Sandven²⁹ and Sullivan, Coleman³⁰, i.e., germ tube formation, carbohydrate fermentation, carbohydrate assimilation, and thermotolerance assay³¹.

8. Statistical Analyses

The values of colony-forming units per mL (cfu/mL) for diabetic and non-diabetic patients were compared, considering independent variables of the study the surfaces collected (mucosa, both sides of the conventional radiographic periapical film, CDR sensor and bite block of the receptor-positioning device) and the simulated radiographic region. The non-parametric Kruskal-Wallis test was used for comparison of the surfaces of collection in diabetic and non-diabetic patients while the Wilcoxon test was used for comparison of the radiographic regions for each type of surface collected. ANOVA and t-test were used for comparison the diabetic and non-diabetic patients with CAL, using BioStat 4.0 software (Belém, PA, Brazil).

RESULTS

The age range of subjects was 18- to 67-years old (male mean = 49.9 ± 0.1 years; female mean = 47.5 ± 1.8 years). Out of 46 participants, 11 (55%) patients without diabetes were female and nine (45%) were male, while 14 (53.9%) diabetic patients were female and 12 (46.1%) were male.

The total distribution of the concentration of glycated hemoglobin was similar in diabetic patients, considering that 25% male and 25% female presented less than 7% (good metabolic control) and 20% male and 30% female presented more than 8% (inadequate metabolic control).

Considering the percentage of patients with CAL ≥ 4 mm, it is possible to note that there was no statistical difference (ANOVA, t-test) between diabetic (male = 63.8%; female = 48.3%) and non-diabetic (male = 76.5%; female = 50.7%) patients.

Considering the presence and quantity of *Candida* species during radiographic examination, it is possible to note that the mucosa followed by the non-sensitive side of the conventional radiographic film showed the statistically highest cfu/mL mean if compared with the others surfaces collected, independently of diabetes presence (Table 1). Considering the different types of

surfaces on simulating regions, it was observed that the upper right molar (Urm) region showed the highest level of colonization during the examination of the receptor-positioning device, the sensor and the non-sensitive film on non-diabetic patients. In diabetic patients, the mucosa of the upper left regions (Ul b and Ul m) showed higher levels of colonization (Table 1).

Table 1 shows statistical differences for the cfu/mL mean, when the collection surface was the conventional radiographic film, on the non-sensitive side, when compared to the oral mucosa in the upper right molar region of diabetic patients. The lower left molars, lower right bicuspid and molar region also demonstrated statistical differences between the oral mucosa

and the receptor-positioning device. There was a statistically significant difference between the colonization of the surface of the device and the sensor for the lower right molar (Kruskal-Wallis, $p < 0.005$).

The mean cfu/mL of non-diabetic patients was statistically different in the oral mucosa and receptor-positioning device for the lower left molar region (Kruskal-Wallis, $p < 0.05$; Table 1). When a Wilcoxon test ($p < 0.05$) was applied to the data of Table 1, a statistical difference was observed in the cfu/mL mean of the upper right molar for the sensitive film surface.

Candida spp. in saliva did not differ significantly between diabetic (mean = 3.0×10^6) and non-diabetic patients

Table 1. Means and standard deviations (in parentheses) of cfu/mL of diabetic patients according to collected surfaces and simulated radiographic region

Simulated region	Surfaces – diabetic					Surfaces – non diabetic				
	Device	Sensor	Sensitive film	Non-sensitive film	Mucosa	Device	Sensor	Sensitive film	Non-sensitive film	Mucosa
Urm	3.7 (11.4)	4.3 (6.6)	2.4 (4.9)	2.7 ^a (7.1)	22.5 ^a (69.8)	4.8 (10.5)	19.1 (23.8)	5.4 (26.0)	15.5 (10.6)	18.2 (39.5)
Urb	7.9 (26.5)	15.6 (41.4)	5.2 (23.2)	8.7 (11.4)	26.8 (75.0)	4.2 (10.3)	10.9 (27.2)	5.4 (28.8)	12.8 (9.4)	73.1 (164.8)
Urc	6.4 (15.6)	1.8 (2.2)	2.6 (12.5)	5.8 (6.9)	43.2 (84.8)	1.5 (4.9)	9.3 (28.0)	3.0 (17.1)	9.2 (7.5)	30.3 (51.9)
Uci	4.2 (13.3)	8.9 (16.7)	2.8 (9.4)	4.0 (6.0)	45.6 (93.5)	0.6 (1.3)	1.7 (2.4)	0.8 (5.5)	3.6 (1.5)	60.4 (125.4)
Ulc	4.3 (14.0)	7.1 (10.9)	3.9 (13.4)	7.1 (9.9)	34.3 (56.8)	1.7 (3.9)	11.3 (30.1)	3.6 (13.4)	6.8 (8.7)	46.1 (75.4)
Ul b	3.6 (5.8)	6.5 (8.3)	8.0 (41.0)	12.1 (18.5)	61.9 (112.8)	0.7 (1.5)	15.3 (42.2)	5.2 (17.8)	7.6 (16.1)	16.0 (41.0)
Ul m	1.9 (4.8)	10.8 (22.0)	4.9 (99.0)	5.1 (11.4)	63.9 (112.4)	0.9 (1.7)	5.9 (8.6)	1.8 (11.1)	6.0 (2.5)	17.2 (32.8)
Llm	2.9 ^b (6.4)	5.9 (8.0)	11.8 (22.8)	9.1 (29.4)	45.6 ^b (75.4)	3.9 ^a (11.1)	14.2 (32.9)	7.5 (23.7)	9.1 (25.0)	56.0 ^a (151.1)
Llb	0.6 (1.2)	5.7 (7.0)	7.7 (23.1)	10.5 (15.9)	21.2 (47.3)	1.4 (4.7)	13.1 (28.8)	3.7 (10.8)	5.4 (7.0)	16.0 (15.9)
Llc	1.5 (5.0)	10.7 (17.8)	8.4 (14.4)	6.2 (21.3)	17.6 (18.8)	1.9 (7.8)	7.2 (20.9)	3.1 (37.1)	12.2 (7.0)	15.3 (16.9)
Lci	1.0 (3.6)	4.3 (8.9)	4.6 (21.8)	5.7 (10.3)	10.6 (27.5)	0.7 (1.7)	10.0 (21.0)	5.6 (19.1)	7.4 (14.8)	15.6 (21.0)
Lrc	6.6 (27.8)	9.7 (26.1)	4.5 (15.6)	6.3 (10.2)	13.0 (43.5)	0.6 (1.7)	8.3 (18.8)	3.7 (9.8)	3.9 (8.9)	14.1 (60.0)
Lrb	0.4 ^c (1.0)	10.0 (22.8)	5.8 (14.2)	5.6 (15.9)	20.7 ^c (48.3)	2.0 (6.7)	3.2 (7.5)	2.9 (2.3)	3.8 (5.2)	9.3 (21.9)
Lrm	1.5 ^{de} (3.5)	22.0 ^d (22.0)	7.8 (23.4)	9.0 (13.8)	39.8 ^e (75.7)	1.7 (3.7)	9.4 (15.6)	4.1 (9.7)	4.6 (5.8)	38.4 (102.2)

Same letters differ statistically (Kruskal-Wallis, $p < 0.05$). Abbreviations: Urm = upper right molar, Urb = upper right bicuspid, Urc = upper right cuspid and lateral incisor, Uci = upper central incisor, Ulc = upper left cuspid and lateral incisor, Ul b = upper left bicuspid, Ul m = upper left molar, Llm = lower left molar, Llb = lower left bicuspid, Llc = lower left cuspid and lateral incisor, Lci = lower central incisor, Lrc = lower right cuspid and lateral incisor, Lrb = lower right bicuspid, Lrm = lower right molar.

Table 2. Frequency of identified species of *Candida* spp., according to the collected surface for diabetic and non-diabetic patients

Species of <i>Candida</i> spp.	Type of collected surface											
	Mucosa		Sensitive film		Non sensitive film		Sensor		Device		Total	
	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND
<i>albicans</i>	93	110	97	84	109	115	65	70	56	52	420	431
<i>krusei</i>	4	5	2	1	-	4	1	1	2	2	9	13
<i>parapsolsis</i>	6	9	4	7	4	5	2	2	5	4	21	27
<i>guillermondi</i>	1	15	4	16	1	14	-	13	2	14	8	72
<i>tropicalis</i>	18	25	10	28	11	32	13	31	-	20	52	136
Total	122	164	117	136	125	170	81	117	65	92	510	679

D = diabetic, ND = non-diabetic.

(mean = 3.8×10^6). Table 2 shows the high prevalence of *Candida albicans* for all collected surfaces from diabetic and non-diabetic patients, followed by *Candida tropicalis*. Tests for the identification of *Candida dubliniensis* were performed, but this specie was not found in any patients. The device surface was the surface that carried the least number of *Candida* colonies and the non-sensitive film for non-diabetics was the surface that presented the highest *Candida albicans* colonization.

Candida albicans was observed in 420 samples, *C. tropicalis* in 52, and *C. parapsilosis* in 21 diabetic and non-diabetic patients for all radiographic regions (Table 2). In this study, 1185 species of *Candida* spp. were identified in the simulated radiographic region for both groups (Table 3). The lower left bicuspid region presented the highest *Candida* colonization (n = 108), whilst the upper central incisor region presented the lowest colonization (n = 67; Table 3).

Table 3. Frequency of identified species of *Candida* spp., according to simulated-radiographic region for diabetics and non-diabetics

Simulated radiographic region		Species of <i>Candida</i>					Total
		<i>albicans</i>	<i>krusei</i>	<i>parapsilosis</i>	<i>guillermondi</i>	<i>tropicalis</i>	
Urm	D	21	1	-	-	-	22
	ND	35	1	2	6	10	54
Urb	D	29	1	4	-	4	38
	ND	35	1	3	6	12	57
Urc	D	31	1	3	1	6	42
	ND	28	-	2	7	11	48
Uci	D	31	1	1	1	3	37
	ND	25	-	-	1	4	30
Ulc	D	33	1	2	1	1	38
	ND	36	1	-	8	7	52
Ulb	D	31	-	-	-	3	34
	ND	34	1	3	8	11	57
Ulm	D	26	-	-	1	3	30
	ND	37	1	1	2	9	50
Llm	D	26	-	-	-	2	28
	ND	35	3	1	7	12	58
Llb	D	37	-	-	1	7	45
	ND	38	3	3	6	13	63
Llc	D	33	-	3	1	10	47
	ND	26	-	2	6	11	45
Lci	D	24	1	2	-	1	28
	ND	24	-	-	9	9	42
Lrc	D	30	-	5	-	4	39
	ND	20	1	2	5	9	37
Lrb	D	33	2	-	-	5	40
	ND	28	-	4	-	9	41
Lrm	D	35	1	-	-	2	38
	ND	30	1	4	1	9	45
Total		851	22	47	78	187	1185

Abbreviations: Urm = upper right molar, Urb = upper right bicuspid, Urc = upper right cuspid and lateral incisor, Uci = upper central incisor, Ulc = upper left cuspid and lateral incisor, Ulb = upper left bicuspid, Ulm = upper left molar, Llm = lower left molar, Llb = lower left bicuspid, Llc = lower left cuspid and lateral incisor, Lci = lower central incisor, Lrc = lower right cuspid and lateral incisor, Lrb = lower right bicuspid, Lrm = lower right molar. D = diabetic, ND = non-diabetic.

DISCUSSION

This study did not confirm the hypothesis that the presence of *Candida* spp. is more frequent in the oral cavity of diabetic than non-diabetic patients, as has been observed in other studies^{9,11,14,15}. This result may be due to the fact that half of the sample had good metabolic control. According to Hill et al.³², diabetes by itself does not put a patient at risk of developing fungal infections, unless his/her metabolic control is poor. It is speculated that there is a tendency towards a greater presence of *Candida* spp. in the oral mucosa of non-diabetic patients in this study. However, due to data analyses of statistical values and considering the extreme values shown by this data set, statistical differences were not observed, with the exception of some regions shown in Tables 2 and 3. This lack of difference suggests that studies about intraoral techniques should consider that the radiographic receptor or the receptor-positioning device may be infected by *Candida* spp. in a similar manner to the patient's oral mucosa, independently of whether the patient is diabetic or not. Often, students that are initiating their studies consider the oral cavity to be potentially infected, but handle the radiographic receptor as if it were not infected.

As regards the distribution of the participants in this study, measurements of glycated hemoglobin suggested that patients who presented glycated hemoglobin of higher than 8% did not have an increased risk of fungal infection. In contrast, a higher occurrence of fungal infections was shown by Hill et al.³² in patients with concentrations of glycated hemoglobin of higher than 12%. The limitation of studies of small sample sizes of patients with diabetes should also be taken into consideration, since such sample sizes may prevent the analysis of risk factors for higher prevalence of fungal infections, such as metabolic control³³.

C. albicans was the most commonly identified candidal species in this and other studies^{12,20,34,35}.

According to Willis et al.²⁰, forty per cent of patients colonized with candidal species had no signs of oral candidosis and a number of candidal species were recovered from the oral cavity of insulin-treated patients. *C. albicans* was the most commonly recovered species, being recovered from 85% of the diabetic patients. *C. dubliniensis* was the second most commonly occurring candidal species. The oral cavity reflects the state of systemic health more frequently than any other part of the body and increased susceptibility to general and oral superficial infections with yeasts has long been associated with diabetes mellitus.

Manfredi et al.¹³ observed that diabetic patients with dentures had more species of *Candida*, with the exception of *Candida albicans*, isolated from their mouths than dentate diabetics. This study showed a higher prevalence of *Candida albicans*, followed by *C. tropicalis* and *C. guilliermondi*. Similarly, a previous study reported a non-significant trend towards a prevalence of species other than *C. albicans* in non-diabetic patients compared to diabetic patients¹³.

In conclusion, there was no difference in colonization (cfu/mL) for the *Candida* spp. between diabetic and non-diabetic patients with periodontal disease, when considering the five collected surfaces and the simulated-radiographic regions studied. *Candida albicans* was the prevalent species of *Candida* spp. found on the collected surfaces and simulated radiographic regions, followed by *Candida tropicalis*.

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REFERENCES

- Bartolini JA, Chariton DG, Flint DJ. Infection control practices in dental radiology. *Gen Dent*. 2003;51:264-71.
- Centers for Disease Control and Prevention. Guidelines for Infection Control in Dental Health-Care Settings — 2003. *MMWR* 2003;52(No. RR-17):[2;31].
- White SC, Glaze S. Interpatient microbiological cross-contamination after dental radiographic examination. *J Am Dent Assoc*. 1978;96:801-4.
- Kalathingal SM, Moore S, Know S, Schuster G, Shrouf MK, Plummer K. An evaluation of microbiologic contamination on phosphor plates in a dental school. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;107:279-82. <http://dx.doi.org/10.1016/j.tripleo.2008.05.025>
- van der Stelt PF. Filmless imaging: the uses of digital radiography in dental practice. *J Am Dent Assoc*. 2005;136:1379-87.
- Hokett SD, Honey JR, Ruiz F, Baisedn MK, Hoen MM. Assessing the effectiveness of direct digital radiography barrier sheaths and finger cots. *J Am Dent Assoc*. 2000;131:463-7.
- Taylor GW, Borgnakke WS. Periodontal disease: associations with diabetes, glycemic control and complications. *Oral Dis*. 2008;14:191-203. <http://dx.doi.org/10.1111/j.1601-0825.2008.01442.x>
- Spolidorio DMP, Boriollo MFG, Estrela C, Spolidorio LC. Diferentes métodos fenotípicos para isolamento e identificação de espécies de *Candida*. *ROBRAC: Rev Odontol Brasil Central*. 2009;18:18-26.
- Lamey PJ, Darwaza A, Fisher BM, Samaranayake LP, Macfarlane TW, Frier BM. Secretor status, candidal carriage and candidal infection in patients with diabetes mellitus. *J Oral Pathol*. 1988;17:354-7. <http://dx.doi.org/10.1111/j.1600-0714.1988.tb01549.x>
- Farah CS, Ashman RB, Challacombe SJ. Oral candidosis. *Clin Dermatol*. 2000;18:553-62. [http://dx.doi.org/10.1016/S0738-081X\(00\)00145-0](http://dx.doi.org/10.1016/S0738-081X(00)00145-0)
- Guggenheimer J, Moore PA, Rossie K, Myers D, Mongelluzzo MB, Block HM et al. Insulin-dependent diabetes mellitus and oral soft tissue pathologies. II. Prevalence and characteristics of *Candida* and candidal lesions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2000;89:570-6. <http://dx.doi.org/10.1067/moe.2000.104477>
- Willis AM, Coulter WA, Sullivan DJ, Coleman DC, Hayes JR, Bell PM, et al. Isolation of *C. dubliniensis* from insulin-using diabetes mellitus patients. *J Oral Pathol Med*. 2000;29:86-90. <http://dx.doi.org/10.1034/j.1600-0714.2000.290206.x>

13. Manfredi M, McCullough MJ, Al-Karaawi ZM, Hurel SJ, Porter SR. The isolation, identification and molecular analysis of *Candida* spp. isolated from the oral cavities of patients with diabetes mellitus. *Oral Microbiol Immunol.* 2002;17:181-5. <http://dx.doi.org/10.1034/j.1399-302X.2002.170308.x>
14. Belazi M, Velegaki A, Fleva A, Gidarakou I, Papanau L, Baka D, et al. Candidal overgrowth in diabetic patients: potential predisposing factors. *Mycoses.* 2005;48:192-6. <http://dx.doi.org/10.1111/j.1439-0507.2005.01124.x>
15. Kumar BV, Padshetty, NS, Bai KY, Rao MS. Prevalence of *Candida* in the oral cavity of diabetic subjects. *J Assoc Phys Indian.* 2005;53:599-602.
16. Diabetes and periodontal diseases. Committee on Research, Science and Therapy. American Academy of Periodontology. *J Periodontol.* 2000;71:664-78. <http://dx.doi.org/10.1902/jop.2000.71.4.664>
17. Christgau M, Palitzsch KD, Schmalz G, Kreiner U, Frenzel S. Healing response to non-surgical periodontal therapy in patients with diabetes mellitus: clinical, microbiological, and immunological results. *J Clin Periodontol.* 1998;25:112-24. <http://dx.doi.org/10.1111/j.1600-051X.1998.tb02417.x>
18. Moore LV, Moore WE, Riley C, Brooks CN, Burmeister JA, Smibert RM. Periodontal microflora of HIV positive subjects with gingivitis or adult periodontitis. *J Periodontol.* 1993;64:48-56. <http://dx.doi.org/10.1902/jop.1993.64.1.48>
19. Lamster IB, Lalla E, Borgnakake WS, Taylor GW. The relationship between oral health and diabetes mellitus. *J Am Dent Assoc.* 2008;139 (Suppl):19S-24S.
20. Willis AM, Coulter WA, Fulton CR, Hayes JR, Bell PM, Lamey P-J. Oral candidal carriage and infection in insulin-treated diabetic patients. *Diabet Med.* 1999;16:675-9. <http://dx.doi.org/10.1046/j.1464-5491.1999.00134.x>
21. Colombo APV, Teles RP, Torres MC, Souto R, Rosalém Jr. W, Mendes MCS et al. Subgingival microbiota of Brazilian subjects with untreated chronic periodontitis. *J Periodontol.* 2002;73:360-9. <http://dx.doi.org/10.1902/jop.2002.73.4.360>
22. Sardi JCO, Duque C, Camargo GACG, Hofling JF, Gonçalves RB. Periodontal conditions and prevalence of putative periodontopathogens and *Candida* spp. in insulin-dependent type 2 diabetic and non-diabetic patients with chronic periodontitis - a pilot study. *Arch Oral Biol.* 2011;56:1098-105. <http://dx.doi.org/10.1016/j.archoralbio.2011.03.017>
23. Melton JJ, Redding SW, Kirkpatrick WR, Reasner CA, Ocampo GL, Venkatesh A, et al. Recovery of *Candida dubliniensis* and other *Candida* species from the oral cavity of subjects with periodontitis who had well-controlled and poorly controlled type 2 diabetes: a pilot study. *Spec Care Dentist.* 2010;30:230-4. <http://dx.doi.org/10.1111/j.1754-4505.2010.00159.x>
24. Armitage G. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol.* 1999;4:1-6. <http://dx.doi.org/10.1902/annals.1999.4.1.1>
25. American Diabetes Association. Standards of medical care in diabetes -2010. *Diabetes Care.* 2010;33(Suppl 1):S11-61. <http://dx.doi.org/10.2337/dc10-S011>
26. Williams DW, Lewis MAO. Isolation and identification of candida from the oral cavity. *Oral Dis.* 2000;6:3-11. <http://dx.doi.org/10.1111/j.1601-0825.2000.tb00314.x>
27. Beighton D, Ludford R, Clark DT, Brailsford SR, Pankhurst CL, Tinsley GF, et al. Use of CHROMagar *Candida* Medium for Isolation of Yeasts from Dental Samples. *J Clin Microbiol.* 1995;33:3025-7.
28. Pfaller MA, Houston A, Coffmann S. Application of CHROMagar *Candida* for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida (Torulopsis) glabrata*. *J Clin Microbiol.* 1996;34:58-61.
29. Sandven P. Laboratory identification and sensitivity testing yeast isolates. *Acta Odontol Scand.* 1990;48:27-36. <http://dx.doi.org/10.3109/00016359009012731>
30. Sullivan D, Coleman D. *Candida dubliniensis*: Characteristics and identification. *J Clin Microbiol.* 1998;36:329-34.
31. Pinjon E, Sullivan D, Salkin I, Shanley D, Coleman D. Simple, inexpensive, reliable method for differentiation of *Candida dubliniensis* from *Candida albicans*. *J Clin Microbiol.* 1998;36:2093-5.
32. Hill LVH, Tan MH, Pereira LH, Embil JA. Association of oral candidiasis with diabetic control. *J Clin Pathol.* 1989;42:502-5. <http://dx.doi.org/10.1136/jcp.42.5.502>
33. Soell M, Hassan M, Miliuskaite A, Haikel Y, Selimovic D. The oral cavity of elderly patients in diabetes. *Diabetes Metab.* 2007;33:S10-8. [http://dx.doi.org/10.1016/S1262-3636\(07\)80053-X](http://dx.doi.org/10.1016/S1262-3636(07)80053-X)
34. Negrato CA, Tarzia O. Buccal alterations in diabetes mellitus. *Diabetol Metab Syndr.* 2010;2:1-11. <http://dx.doi.org/10.1186/1758-5996-2-3>
35. Manfredi M, McCullough MJ, Vescovi P, Al-Kaarawi ZM, Porter SR. Update on diabetes mellitus and related oral diseases. *Oral Dis.* 2004;10:187-200. <http://dx.doi.org/10.1111/j.1601-0825.2004.01019.x>

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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