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Short-term comparison between clinical and microbial profiles in diabetic and non-diabetic patients with chronic periodontitis after non-surgical periodontal therapy

Comparação a curto prazo entre perfis clínicos e microbianos em pacientes diabéticos e não diabéticos com periodontite crônica após terapia periodontal não cirúrgica

Comparación a corto plazo entre perfiles clínicos y microbianos en pacientes diabéticos y no diabéticos con periodontitis crónica después de la terapia periodontal no quirúrgica

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Abstract
Aim: To compare clinical and microbial profiles in type 2 diabetic (DM) and non-diabetic (NDM) patients with chronic periodontitis after non-surgical periodontal therapy. Methods: 30 subjects with periodontitis were separated into two groups: 15 DM and 15 NDM. Blood parameters and clinical parameters were assessed. Subgingival biofilm samples were collected from periodontal pockets (PD > 5mm) and ckecked by the presence of *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Prevotella intermedia* (Pi), *Campylobacter rectus* (Cr), *Candida albicans* (Ca), *Candida glabrata* (Cg), *Candida tropicalis* (Ct), *Candida dublinienses* (Cd) by Polymerase Chain Reaction. Data were statistically analyzed, considering $p < 0.05$. Results: PI, PD, and CAL significantly reduced after 3 months of periodontal therapy in both groups. Only levels of HbA1c reduced in the DM group after 3 months. Comparisons between baseline and 3 months after periodontal therapy revealed a reduction of Aa, Pg, Tf, Pi, Ct and Cd for the NDM group and Aa, Pg, Tf, and Ca for the DM group. Conclusions: The periodontal therapy was effective in reducing clinical parameters and microbial levels, regardless the presence of the diabetes mellitus. However, high levels of Candida ssp. remained in periodontal pockets of diabetic patients even after therapy.
Keywords: Periodontitis; Periodontal disease; Candida; Glycemic control; Type 2 diabetes mellitus.

Resumo
Objetivo: Comparar o perfil clínico e microbiano em pacientes diabéticos tipo 2 (DM) e não diabéticos (NDM) com periodontite crônica após terapia periodontal não cirúrgica. Métodos: 30 indivíduos com periodontite foram separados em dois grupos: 15 DM e 15 NDM. Parâmetros sanguíneos e parâmetros clínicos foram avaliados. Amostras de biofilme subgengival foram coletadas de bolsas periodontais (PD > 5mm) e verificadas pela presença de *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Prevotella intermedia* (Pi), *Campylobacter rectus* (Cr), *Candida albicans* (Ca), *Candida glabrata* (Cg), *Candida tropicalis* (Ct), *Candida dublinienses* (Cd) por Reação em Cadeia da Polimerase. Os dados foram analisados estatisticamente, considerando $p < 0,05$. Resultados: PI, PD e CAL reduziram significativamente após 3 meses de terapia periodontal em ambos os grupos. Apenas os níveis de HbA1c reduziram no grupo DM após 3 meses. Comparações entre a linha

de base e 3 meses após a terapia periodontal revelaram uma redução de Aa, Pg, Tf, Pi, Ct e Cd para o grupo NDM e Aa, Pg, Tf e Ca para o grupo DM. Conclusões: A terapia periodontal foi eficaz na redução dos parâmetros clínicos e dos níveis microbianos, independentemente da presença do diabetes mellitus. No entanto, altos níveis de Candida ssp. permaneceram nas bolsas periodontais de pacientes diabéticos mesmo após a terapia.
Palavras-chave: Periodontite; Doença periodontal; Candida; Controle glicêmico; Diabetes mellitus tipo 2.

Resumen
Objetivo: Comparar los perfiles clínicos y microbianos en pacientes diabéticos tipo 2 (DM) y no diabéticos (NDM) con periodontitis crónica después de la terapia periodontal no quirúrgica. Métodos: 30 sujetos con periodontitis fueron separados en dos grupos: 15 DM y 15 NDM. Se evaluaron parámetros sanguíneos y parámetros clínicos. Se recolectaron muestras de biopelícula subgingival de bolsas periodontales (PD > 5 mm) y se comprobó la presencia de *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Prevotella intermedia* (Pi), *Campylobacter rectus* (Cr), *Candida albicans* (Ca), *Candida glabrata* (Cg), *Candida tropicalis* (Ct), *Candida dublinienses* (Cd) por Reacción en Cadena de la Polimerasa. Los datos fueron analizados estadísticamente, considerando $p < 0,05$. Resultados: PI, PD y CAL se redujeron significativamente después de 3 meses de terapia periodontal en ambos grupos. Solo los niveles de HbA1c se redujeron en el grupo DM después de 3 meses. Las comparaciones entre el inicio y 3 meses después de la terapia periodontal revelaron una reducción de Aa, Pg, Tf, Pi, Ct y Cd para el grupo NDM y Aa, Pg, Tf y Ca para el grupo DM. Conclusiones: La terapia periodontal fue efectiva en la reducción de parámetros clínicos y niveles microbianos, independientemente de la presencia de diabetes mellitus. Sin embargo, los altos niveles de Candida ssp. permaneció en las bolsas periodontales de los pacientes diabéticos incluso después de la terapia.
Palabras clave: Periodontitis; Enfermedad periodontal; Candida; Control glicémico; Diabetes mellitus tipo 2.

1. Introduction

Type 2 diabetes mellitus (DM) is a metabolic disorder, characterized by hyperglycemia due to defective insulin function or altered insulin cell receptors (insulin resistance) rather than its deficiency (Roden, 2016). Hyperglycemia can cause progressive microvascular complications, microangiopathy, nephropathy, neuropathy, macrovascular disease, and delayed wound healing (Mealey & O'Campo, 2007). In addition, DM is a recognized risk factor for periodontal disease, being able to promote its progression (American Diabetes Association, 1997; Wu et al., 2020).

Periodontitis is a common inflammatory disease, induced by bacteria, characterized by the destruction of periodontal tissues and loss of connective tissue attachment, and its risk is increased by approximately three times in diabetic patients (greater risk of other macrovascular and microvascular complications) compared to non-diabetics (Lee et al., 2019). The effect of periodontitis on diabetes may be related to the penetration of the host tissues by bacteria or their degradation products into the systemic circulation. Activation of an exaggerated systemic inflammatory response to subgingival bacteria leads to an acute phase protein burst and systemically elevated levels of proinflammatory mediators which facilitate insulin resistance (Acharya et al., 2018). On the other hand, the effect of diabetes on periodontitis, is that if it is not controlled, it influences the expression of enzymes that degrade inflamed gingival tissue (Bastos et al., 2017).

Regarding the composition of the microbiota in diabetic patients, there is still some controversy. Some studies have shown similar microbial profiles in diabetic patients compared to those without diabetes (Kocak et al., 2020). However, other studies have demonstrated that diabetic patients present a higher prevalence of *A. actinomycetemcomitans, Campylobacter spp., Treponema. denticola, Tannerella forsythia, Porphyromonas gingivalis, and Prevotella. nigrescens* (Ebersole et al., 2008; Campus et al., 2005). Considering the presence of *Candida* ssp., the incidence of infections caused by these fungi is higher in immunocompromised patients, including diabetes mellitus. *Candida albicans* is the most frequently isolated and has greater virulence in diabetic patients (Hintao et al., 2007; Gomes et al., 2017). In addition, it may be involved in the pathogenesis of periodontal disease, favoring the increase in the pathogenic microorganisms due to its influence on the host defense mechanisms (Pérez-Losada et al., 2016).

Periodontal treatment consists of scaling and root planning (SRP), alone or associated with antiseptics, antimicrobials, photodynamic therapy, or surgical procedures (Joseph et al., 2017). The scaling and root planning might promote clinical

benefits in terms of reducing inflammation, decreasing probing pocket depth, and attachment level measurements, especially at deeper sites (American Diabetes Association, 1997). The goals of periodontal therapy are to alter or eliminate the microbial etiology and contributing risk factors for periodontitis, thereby arresting the progression of the disease, and restoring the health, comfort, and function with appropriate esthetics (American Diabetes Association, 2016).

Diabetes and periodontal disease seem to have a bidirectional relationship. Altered systemic inflammatory response has been recognized in both periodontal disease and DM (Kocher et al., 2018). Some studies have shown that periodontal therapy may reduce inflammatory conditions, resulting in improvement in metabolic control. However, there is no clear evidence of a relation between periodontal treatment and improvements in glycemic control in patients with type 2 diabetes mellitus (Pérez-Losada et al., 2016), needing further studies. Therefore, the aim of the study was to compare clinical and microbial profiles in type 2 diabetics (DM) and non-diabetics (NDM) with chronic periodontitis after non-surgical periodontal therapy.

2. Methodology

Study population

This study was conducted at the Fluminense Federal University, Nova Friburgo, Rio de Janeiro State, Brazil. Prior to participation, the purpose and procedures were fully explained to all patients, who consequently gave written informed consent in accordance with the Helsinki Declaration. Fifty-nine subjects with moderate to severe periodontitis, separated in two groups; 15T2DM and 15 NDM, with mean age ranges NDM = 52.33± 12.02 and DM = 54.13± 9.72 years, were recruited from the Department of Periodontology, School of Dentistry, for this single blind study. Patients with type 2 diabetes were diagnosed by an endocrinologist according to the criteria published by the American Diabetes Association (1997). Characteristics of the subjects, including age, sex, ethnicity, time of diabetes, and medical and dental histories were taken, and the clinical evaluation was performed at prescreening visits.

Inclusion criteria for acceptance in this study were based on in the periodontal diagnosis of the participants was based on the 2017 World Workshop on the Classification of Periodontal (Jepsen et al., 2018). Both groups DM and NDM including generalized moderate-to-severe periodontitis, patients with GI > 1, >30% of sites with PPD of ≥5 mm, positive bleeding on probing (BOP), CAL ≥5 mm with radiographic evidence of bone loss. The glycemic status of patients previously diagnosed with type 2 DM was confirmed by their glycated hemoglobin A1c (HbA1c) and fasting glucose levels - FGL (American Diabetes Association, 2014). Patients were categorized into two groups (n=15 each) on the basis HbA1c and FGL levels. Group NMM: HbA1c < 6.5% and FGL levels <100 mg/dL and DM group with HbA1c levels > 6.5% and FGL levels ≥126 mg/dL.

The participants were required not to have undergone periodontal treatment or professional cleaning of the teeth for at least 1 year prior to the study. Exclusion criteria were patients with systemic diseases; diabetes; osteoporosis; pregnant; use of immune suppressive medication, phenytoin, cyclosporine, calcium channel blockers, or any use of antibiotics or nonsteroidal anti-inflammatory drugs in the past 3 months; and any medical conditions requiring immunotherapy or diagnosed as HIV+ or with AIDS, that could interfere with the periodontium.

Blood samples were collected in vacuum tubes in the morning for determination of fasting glucose levels (FGL), glycosylated hemoglobin (HbA1c), triglycerides (TRG), High–Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) levels at a specialized clinical laboratory in the same city.

An experienced periodontist (GACGC) evaluated the clinical parameters and samples collected. The following periodontal parameters were recorded: Plaque index (PI), presence and absence of bleeding on probing (BOP), probing depth

(PD), gingival recession (GR), and clinical attachment level (CAL) were recorded in millimeters, using a periodontal probe PCP15 (PCP-UNC15, Hu-Friedy, Chicago, IL) at six sites. Sites with a probing depth (PD) >3mm in a minimum of two teeth in different arches were selected and labeled to receive subgingival treatment and sample collections.

After clinical measurements, the supragingival biofilm was removed with sterile gauze. Subgingival samples were taken with sterile paper points from the deepest pocket PD (>5mm) for 30s in each patient. Pooled biofilms were stores in Eppendorf tubes containing Tris-EDTA and were stored at -200C. Samples were analyzed microbiologically by polymerase chain reaction (PCR). After removing baseline samples of biofilm, the scaling and root planning were performed using ultrasonic devices and manual Gracey curettes (Hu-Friedy®, Chicago USA). SRP was scheduled in one or two sessions 1 week apart according to the periodontal disease severity and the number of teeth present. Oral hygiene instructions for home care procedures (Modified Bass brushing technique, interproximal cleaning, and use of tongue scrapers) were given by one experienced periodontist. The maintenance therapy included professional plaque control at 1-month intervals during the 3 months of the study. All parameters were recorded at baseline and 3 months post-therapy.

Microbiological Assessment

The primers (Gibco BRL-Life technologies of Brazil Ltda. São Paulo, SP, Brazil) evaluated and used in this study are listed in Table 1.

Table 1. Primers used in the PCR analysis.

	Primer pairs (5´-3´)	Thermal conditions	Amplicon length (bp)	Reference
16S Univ*	5´AGA GTT TGA TCC TGG CTC AG 3´ 5´AAG GAG GTG ATC CAG CC 3´	30 cycles – 94ºC for 30 s, 55ºC for 5seg, 72ºC for 2 min	1500	Ashimoto *et al*., 1996
Aa	5´AAA CCC ATC TCT GAC TTC TTCTTC 3´ 5´ATG CCA ACT TGA CGT TA AT 3´	36 cycles – 94ºC for 30 s, 55ºC for 1 min, 72ºC for 2 min	557	Slots *et al.*, 1995
Pg	5´AAT CGT AAC GGG CGA CAC AC 3´ 5´GGG TTG CTC CTT CAT CAT AC 3´	43 cycles – 95ºC for 45 s, 54ºC for 45 s, 72ºC for 1 min	593	Sardi *et al.*, 2012
Tf	5´GCG TAT GTA ACC TGC CCG CA 3´ 5´TGC TTC AGT GTC AGT TAT ACC T3´	46 cycles – 95ºC for 30 s, 60ºC for 1 min, 72ºC for 1 min	641	Benkirane *et al*., 1995
Pi	5´TTTGTTGGGGAGTAAAGCGGG3´ 5´TACACATCTCTGTATCCTGCGT3´	36 cycles – 94ºC for 30 s, 55ºC for 1 min, 72ºC for 2 min	575	Ashimoto *et al*., 1996
Cr	5´TTTCGGAGCGTAAACTCCTTTTC3´ 5´TTTCTGCAAGCAGACACTCTT3´	36 cycles – 94ºC for 30 s, 55ºC for 1 min, 72ºC for 2 min	595	Ashimoto *et al*., 1996
Ca	5´ACT GCT CAA ACC ATC TCT GG 3´ 5´CAC AAG GCA AAT GAA GGA AT 3´	38 cycles – 94ºC for 1 min, 53º C for 1 min and 72ºC for 30 s	452	Sardi *et al.*, 2012
Cd	5´ GTA TTT GTC GTT CCC CTT TC 3´ 5´ GTG TTG TGT GCA CTA ACG TC 3´	38 cycles – 94ºC for 1 min, 54 ºC for 1 min and 72 ºC for 30 s	288	Donnelly *et al.*, 1999
Ct	5´CAC CCA AAC AAT TAC CAA GT 3´ 5´ TGC AAA CTC TTT ACC TGG AT 3´	36 cycles – 94 ºC for 1 min, 51 ºC for 1 min and 72 ºC for 30 s	253	Sardi *et al.*, 2012
Cg	5´ GGA GAT AGA CTG GGC GTT AT 3´ 5´ GTT GTT CAA TGG CTT TCT TC 3´	30 cycles – 94 ºC for 1 min, 56 ºC for 1 min	314	Sardi *et al.*, 2012

16S Universal Primer (16S Univ), Aggregatibacter actinomycetemcomitans (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Prevotella intermedia* (Pi), *Campylobacter rectus* (Cr), *Candida albicans* (Ca), *Candida glabrata* (Cg), *Candida tropicalis* (Ct), *Candida dublinienses* (Cd). Source: Authors (2022).

Tests were performed to verify primer specificity. For this purpose, PCR reactions with specific primers for *A. actinomycetemcomitans* (Aa), *P. gingivalis* (Pg), *T. forsythia* (Tf), *Prevotella intermedia* (Pi), *Campylobacter rectus* (Cr), *Candida albicans* (Ca), *Candida glabrata* (Cg), *Candida tropicalis* (Ct), *Candida dublinienses* (Cd) were processed.

The PCR conditions were previously published by Klein and Gonçalves (2003). For PCR, 36.25µl of sterile distilled water was added to the clinical samples. The samples were dispersed by vortex for 1 minute and then boiled for 10 minutes. The PCR was processed using 5.0µl of sample added to 45µl of reaction buffer containing 1.5mM MgCl2 (Taq DNA polymerase), 200µM dNTP (dNTP mixture), 2µM of each primer, and 2 u Taq DNA polymerase. In addition to the samples, positive and negative controls were used in each experiment. PCR amplification was performed as previously described under the following conditions [Table 1]. The PCR products were analyzed by electrophoresis in 1.5% agarose gel using Tris-borate-EDTA buffer. A 100bp or 1 Kb DNA ladder was included in each gel. The DNA was stained with ethidium bromide and visualized under ultraviolet illumination.

Statistical Analysis

Statistical tests were performed using SPSS 17.0 software (IBM Corp., USA) to compare the results between the NDM and DM groups considering baseline and 3 months after periodontal therapy. Paired or unpaired Student t-tests were performed to compare age, sex, ethnicity, time of diabetes, FGL, HbA1c, TRG, HDL, LDL, PI, BOP, PD, GR, and CAL between the groups. DM and NDM microbial profiles including the percentages of periodontopathogenic bacteria and *Candida*

spp. were compared using the unpaired Student t-test. Statistical significance was defined when p values were lower than 0.05.

3. Results

Blood samples and clinical parameters

At baseline, 30 subjects (15 DM and 15NDM) were evaluated. No statistical differences were found between DM and NMD groups at baseline considering age, sex, ethnicity, and time of diabetes. The mean duration of diabetes was 7.93 years (SD = 8.1) [Table 2]. FGL, Hba1c, LDL, HDL, and TRG values were analyzed before and after periodontal treatment [Table 3]. Statistical higher levels of FGL and Hba1c were observed for DM in comparison to NDM at baseline. After 3 months, only Hba1c reduced in the DM group.

Table 2. Characteristics of the participants.

	NDM	DM
	(n= 15)	(n= 15)
Age (means± SD) (years)	52.33 ±12.02	54.13 ± 9.72
Sex n (%)		
Female	8 (53.3)	6 (40)
Male	7 (46.7)	9 (60)
Ethnicity n(%)		
White	7 (46.7)	11 (73.3)
Afro-american	5 (33.3)	3 (20)
Black	3 (20)	1 (6.7)
Time of diabetes (means± SD) (years)	NA	7.93 ± 8.1

* Statistical difference between DM and NDM groups for baseline (unpaired Student t-test; $p < 0.05$). Source: Authors (2022).

Table 3. Glycemic and lipic conditions (means± standard deviations) of DM and NDM patients at baseline and 3 months after periodontal therapy.

	NDM		DM	
	Baseline	3 months	Baseline	3 months
	(n= 15)	(n= 15)	(n= 15)	(n= 15)
FGL (mg/dl)	89.13 ±11.95§	90.06 ± 10.2#	134.8 ± 58.8	145.6 ± 65.85
HbA1c (%)	4.77 ± 0.59§	4.95 ± 0.47#	7.74± 1.87*	5.72 ± 2
LDL (mg/dl)	120.66 ± 36.91	117.53 ± 30.95	125.52 ± 21.29	128.2 ± 25.72
HDL(mg/dl)	46.6 ±12.4	49.93 ± 12.74	43.13±6.1	46.53 ± 9.5
TGR (mg/dl)	143.86 ± 114.4	101.73 ± 44.51#	164.93 ± 106.99	170.93 ± 83.8

* Statistical difference between baseline and 3 months (paired Student t-test; $p<0.05$). § Statistical difference between NDM and DM groups for baseline (unpaired Student t-test; $p <0.05$). # Statistical difference between NDM and DM groups for 3 months (unpaired Student t-test; $p <0.05$). Source: Authors (2022).

Periodontal parameters from DM and NDM patients can be observed in Table 4. Patients presented moderate to severe periodontal disease (means PD=5.64mm and CAL=7.45 mm for DM and means PD=5.24mm and CAL=6.41 mm for NDM group) with significant BOP (means BOP=47.86% for DM and means BOP=25.24% for NDM group) and no good plaque control (means PI=64.79% for DM and mean PI=32.54% for NDM group). There were statistical differences between NDM and DM patients for PI, BOP and CAL at baseline. These patients were treated with non-surgical periodontal treatment

associated with hygiene control. Both groups showed periodontal response to treatment since PI, PD, and CAL significantly reduced after 3 months of nonsurgical periodontal treatment for both groups. In the DM group, BOP also statistically reduced with the treatment.

Table 4. Periodontal conditions (means±standard deviations) of DM and NDM patients at baseline and 3 months after periodontal therapy.

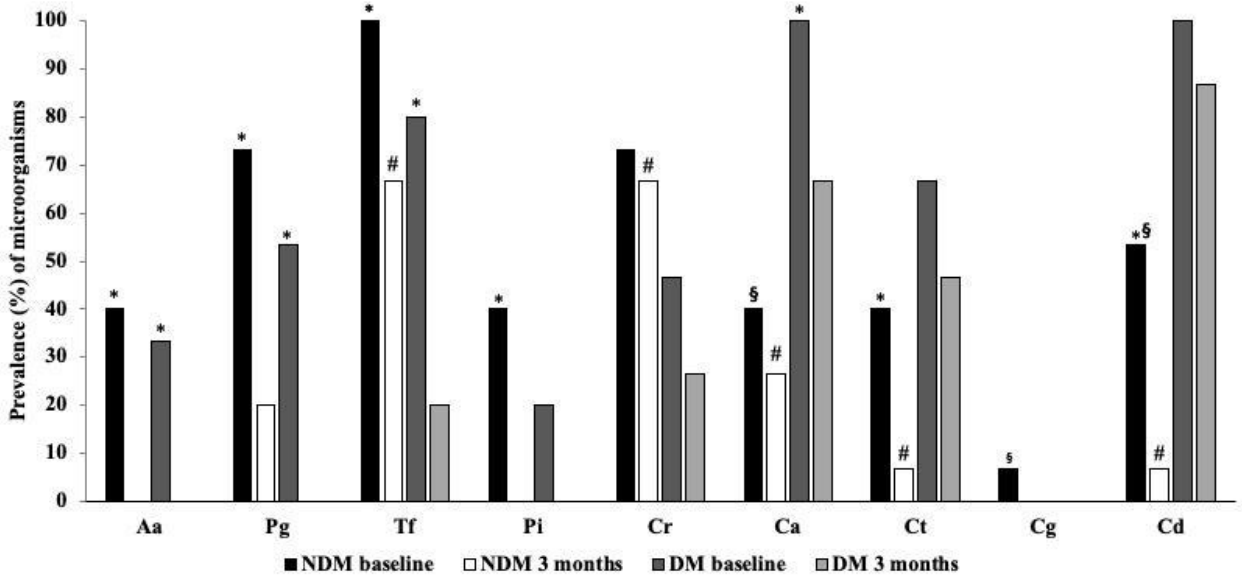
	NDM			DM		
	Baseline (n= 15)	3 months (n= 15)	**Indexes Reduction**** Baseline x 3 months (n=15)	Baseline (n= 15)	3 months (n= 15)	**Indexes Reduction**** Baseline x 3 months (n=15)
PI (%)	32.54±28.43*§	15.56±14.47	-16.98(19.47)‡	64.79 ±23.22*	15.48±13.98	-49.31(17.56)
BOP (%)	25.24 ±23.22§	14.17±10.62	-11.07(17.83)‡	47.86±22.80*	16.63±14.81	-31.23 (19.35)
PD (mm)	5.24 ±0.47*	3.2±0.72	-2.03(0.51)‡	5.64±0.81*	2.95±0.72	-2.68(0.84)
GR (mm)	1.16± 1.61	1.43± 1.61	0.26(0.46)	1.80±1.27	2.11±1.56	0.32(0.44)
CAL (mm)	6.41 ±1.88*§	3.79± 1.37	-1.83(0.74)‡	7.45±1.87*	4.34±1.75	-2.37(0.75)

* Statistical difference between baseline and 3 months (paired Student t-test; p<0.05). § Statistical difference between NDM and DM groups for baseline (unpaired Student t-test; p <0.05). # Statistical difference between NDM and DM groups for 3 months (unpaired Student t-test; p <0.05). ‡ Statistical difference between NDM and DM groups considering indexes reduction (unpaired Student t-test; p <0.05). **Means (standard error) of the differences between baseline and 3 months of treatment for each group of patients (NDM and DM). Plaque index (PI), bleeding on probing (BOP), pocket probing depth (PPD), gingival recession (GR), clinical attachment level (CAL), fasting glucose levels (FGL), glycosylated hemoglobin (HbA1c), triglycerides (TRG), High–Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL). Source: Authors (2022).

Microbiological Parameters

Figure 1 shows the percentages of sites (with PD ≥5 mm) with *A. actinomycetemcomitans* (Aa), *P. gingivalis* (Pg), *T. forsythia* (Tf), *P. intermedia* (Pi), *C. rectus* (Cr), *C. albicans* (Ca), *C. glabrata* (Cg), *C. tropicalis* (Ct), and *C. dublinienses* (Cd) at baseline and after 3 months of periodontal therapy in the DM and NDM groups. At baseline, *Ca* levels were significantly higher in the NDM group compared to the DM, and no significant differences were found between the groups for any bacteria tested. After 3 months of periodontal therapy, only *Tf* and *Cr* levels were significantly higher in the NDM group compared to the DM group. Otherwise, at follow up, levels of *Ca*, *Ct and Cd* were higher for DM group. Comparisons between baseline and 3 months after periodontal therapy showed significant reductions in *Aa, Pg, Tf, Pi, Ct* and *Cd* percentages for NDM group. The levels of the microorganisms *Aa, Pg, Tf,* and *Ca* statistically reduced for DM groups, after 3 months compared to baseline (Figure 1).

Figure 1. Prevalence of putative periodontal pathogens and *Candida* spp. in DM and NDM patients at baseline and 3 months after periodontal therapy.



* Statistical difference between baseline and 3 months (paired Student t-test; $p<0.05$). § Statistical difference between NDM and DM groups at baseline (unpaired Student t-test; $p <0.05$). # Statistical difference between NDM and DM groups after 3 months (unpaired Student t-test; $p <0.05$). *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Prevotella intermedia* (Pi), *Campylobacter rectus* (Cr), *Candida albicans* (Ca), *Candida glabrata* (Cg), *Candida tropicalis* (Ct), *Candida dublinienses* (Cd). Source: Authors (2022).

4. Discussion

Hyperglycemia induces changes in the oral microbiome since poor glycemic control is associated with increased levels and frequencies of periodontal pathogens in the subgingival biofilm of diabetic patients. A bidirectional relationship between periodontal diseases and diabetes mellitus (DM) may occur, as DM patients may have an exacerbated inflammatory response, deficient repair and bone resorption that aggravates periodontal disease, while higher levels of systemic pro-inflammatory mediators found in patients with severe disease periodontal increases insulin resistance (Negrini et al., 2021). In the current study, clinical and microbial profiles were different between patients with DM and NDM at baseline, however, both parameters improved with non-surgical periodontal therapy for the groups, with some few differences between them after 3 months, mainly related to prevalence of some periodontopathogens and *Candida* spp.

Overall, improvement in clinical parameters (PI, BOP, PD, GR and CAL) in the present study was observed for both groups after treatment, showing that basic periodontal therapy associated with oral hygiene control measures is efficient, from biofilm removal by scaling and root planning (SRP), being essential and even more relevant in patients for whom periodontal infection constitutes a health risk, such as those with diabetes mellitus (Lee et al., 2020), highlighting what evidence has always indicated, that non-surgical periodontal treatment is the gold standard of periodontal treatment (Raman et al., 2014). Lalla et al. (2007) reported a 50% reduction in clinical parameters from baseline and after periodontal treatment. A study conducted in China demonstrated that non-surgical therapy for periodontitis was beneficial both in maintaining periodontal health and in reducing blood glucose levels in patients with type 2 DM and chronic periodontitis (Yun et al., 2007). As basic periodontal treatment is well established in the literature, the present findings agree with previous studies, in which diabetics and non-diabetics did not differ in periodontal healing in the short term after non-surgical treatment (Yun et al., 2007; Navarro-Sanchez et al., 2007). A metanalysis and systematic review showed that periodontal therapy was able to reduce the level of serum inflammatory factors, which in turn may benefit glycemic control (Graziani et al., 2018).

In the present study, elevated levels of *A. actinomycetemcomitans* (Aa), *P. gingivalis* (Pg), *T. forsythia* (Tf), *P. intermedia* (Pi) and *C. rect*us (Cr) were found for both DM and NDM groups, at baseline and three months after therapy, without difference between them. However, for both groups of patients, periodontal therapy significantly reduced the majority of the microorganisms tested. These findings are similar to those obtained by Sbordone et al. (1990) and Kocak et al. (2020), who found no significant differences in the presence of subgingival periodontal bacteria in both groups. In contrast, Ebersole et al. (2008) reported a higher prevalence of *P. gingivalis*, *A. actinomycetemcomitans* and *Campylobacter* spp, in the subgingival plaque of patients with DM. In another study, diabetic individuals had higher levels of *T. denticola*, *S. sanguinis*, *P. nigrescens*, *S. intermedia* and *S. oralis* in the supragingival plaque (Hintao et al., 2007). *P. gingivalis* was detected more frequently in individuals with increased HbA1c values, mainly with type II fimbriae, while improvements in HbA1c values were only observed in individuals without type II clones (Makiura et al., 2008). The persistence of certain *P. gingivalis* phenotypes may influence glycemic control in patients with type 2 diabetes (Campus et al., 2005).

At baseline, the presence of *Candida* ssp. in periodontal pockets, mainly *C. albicans*, followed by *C. dublinienses*, was statistically higher in the DM group than in the NDM group. This result suggests that diabetic patients have more fungi in the periodontal pocket than normoglycemic patients, similar to the results obtained by Gomes et al. (2017). These authors evaluated 30 biofilm samples collected from periodontal pockets and 13 had positive cultures for *C. albicans*, 77% in diabetic patients and 23% in normoglycemic patients. A larger amount (87.5%) of *C. albicans* strains was detected in the oral cavity of diabetic patients, while in patients without diabetes this rate dropped to 50% (Premkumar et al., 2014), indicating a positive correlation between glycemic control and *Candida* colonization (Lydia et al., 2016). *C. albicans*, *C. dubliniensis*, *C. tropicalis* and *C. glabrata* were detected in 57%, 75%, 16% and 5% of periodontal pockets in DM patients, respectively. In non-diabetic patients, *C. albicans* and *C. dubliniensis* were present in 20% and 14%, respectively (Sardi et al., 2012). In another study, the most frequently isolated species was *C. albicans* followed by *C. glabrata* and *C. tropicalis* (Matic et al., 2019). Al Mubarak et al. (2013) observed that overall prevalence of *Candida* in diabetic patients with periodontitis was 52% and the most common species identified were *C. albicans* (38%), followed by *C. dubliniensis* (9.5%), *C. tropicalis* (4.7%) and *C. glabrata* (4.7%), similar to the current study.

After 3 months, some species of *Candida*. (*C. albicans, C. tropicalis* and *C. dublinienses*) remained higher in periodontal pockets of patients with DM, indicating that these species may be resistant to traditional periodontal therapies. Several mechanisms are attributed to the higher prevalence of *Candida* sp. in patients with DM depending on local or systemic factors (Rodrigues et al., 2019). The increased colonization by *Candida* in diabetic patients can be attributed to greater adherence of fungi to epithelial cells, facilitated by increased glucose content in saliva, genetic susceptibility to infection, altered cellular and humoral immune defense mechanisms and local factors. The higher incidence of *Candida* in diabetic patients can be also explained by the fact that the oral microbiome is altered by endocrine abnormalities in diabetes mellitus (Rodrigues et al., 2019; Mealey & Rose, 2008). It is relevant to consider that blood glucose levels (FGL) did not change after treatment, suggesting that periodontal pockets remained a favorable environment for the persistence of *Candida.*

5. Conclusion

Considering short-term of evaluation, non-surgical periodontal therapy was effective in controlling clinical and microbial parameters in diabetic and non-diabetic patients with chronic periodontitis. However, high levels of *Candida* ssp. remained in periodontal pockets of diabetic patients even after periodontal therapy.

Considering that this present study was limited by the small sample and the short period of evaluation, other longitudinal studies should be carried out analyzing the effect of traditional and alternative therapies in the presence of

Candida spp. and periodontopathogens and the influence of effective glycemic control, since periodontal therapeutic interventions have been associated with better glycemic control in patients with diabetes mellitus.

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