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ORIGINAL ARTICLE

Microbiological profile associated with peri-implant diseases in individuals with and without preventive maintenance therapy: a 5-year follow-up

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Abstract

Objectives Clinical and microbiological longitudinal changes in individuals with peri-implant mucositis (PM) with or without preventive maintenance therapy (PMT) have not been reported, especially in long periods of monitoring. This 5-year follow-up study aimed to assess the clinical and microbiological changes along time in individuals initially diagnosed with PM.

Materials and methods Eighty individuals diagnosed with PM (T1) and followed during 5 years (T2) were divided into one group with PMT during the study period (GTP; $n = 39$) and another group without PMT (GNTP; $n = 41$). Full-mouth periodontal/peri-implant examinations were performed. Peri-implant microbiological samples were collected and analyzed through qPCR for *Tannerella forsythia*, *Treponema denticola*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Actinomyces naeslundii* at T1 and T2.

Results GNTP presented higher incidence of peri-implantitis than GTP. Moreover, GNTP showed significantly higher total bacterial load and higher frequency of the evaluated orange complex bacteria than GTP. Individuals who progressed to peri-implantitis presented significantly higher total bacterial load and higher frequencies of *P. gingivalis*, *T. denticola*, and *F. nucleatum*.

Conclusions The absence of regular appointments for PMT was associated with a higher incidence of peri-implantitis and a significant increase in total bacterial load.

Clinical relevance Regular visits during PMT positively influenced subgingival microbiota and contributed to peri-implant homeostasis and clinical status stability during a 5-year monitoring period. Compliance with PMT programs should be reinforced among individuals rehabilitated with dental implants.

Keywords Peri-implant mucositis · Peri-implantitis · Periodontitis · Microbiological · Maintenance

Introduction

Peri-implant mucositis (PM) is a reversible inflammatory condition whose main clinical characteristic is bleeding on probing. Erythema, swelling, and/or suppuration may also be present. On the other hand, peri-implantitis (PI) is an irreversible plaque-associated pathological condition occurring in tissues around dental implants, characterized by inflammation in the peri-implant mucosa and subsequent progressive loss of supporting bone [1].

Regular appointments for preventive maintenance therapy (PMT) aim to maintain the health of peri-implant tissues in long term [2, 3]. In a systematic review of nine studies, no evidence of an appropriate frequency for PMT visits was determined [4]. However, regular appointments have been classified as those visits with intervals up to 1 year [5]. In recent studies, the lack of regularity in PMT visits was identified as a risk factor for the occurrence of PI [6–9]. Furthermore, the concept of reversibility of PM is still not fully clear. Experimental PM in men showed a healing time longer than

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3 weeks to achieve pre-experimental levels of mucosal health after plaque control was reinstituted [10]. This topic underscores the need for PMT and rigorous plaque control for patients with PM or even peri-implant health.

Subgingival microbioma studies revealed that a group of bacteria, especially *Tannerella forsythia*, *Treponema denticola*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Prevotella nigrescens*, *Eubacterium nodatum*, and *Peptostreptococcus micros*, have an important role in periodontitis (PE) [11–13].

Additionally, microbiological reviews have shown that the peri-implant microbioma, whether in health or disease, is similar to that around teeth [13–17]. Periodontal pathogens found in sites with PE are often found in implants with PI [16–18]. An increase in bacteria counts such as *T. forsythia*, *T. denticola*, *P. gingivalis*, *P. intermedia*, and *F. nucleatum* was identified in implants with PI [19–22]. Cross-sectional and case-control studies suggested that the microbiota around implants with PI is much more complex and diverse than that found in teeth with PE [17, 18, 23–25]. However, few longitudinal studies have assessed changes in peri-implant microbiota [26, 27]. It is important to notice that some assessments were performed only for short periods of time (up to 12 months).

Although some studies with different methodologies [24, 28, 29] reported findings on microbiological peri-implant diseases, to the best of our knowledge, clinical and microbiological longitudinal changes in individuals under PM in the absence or presence of PMT have not been reported, especially in long periods of time.

Hence, the objective of the present study was to evaluate, in a longitudinal period of 5 years, the peri-implant condition and the differences in the frequencies of *T. forsythia*, *T. denticola*, *P. gingivalis*, *P. intermedia*, *F. nucleatum*, and *Actinomyces naeslundii* in individuals initially diagnosed with PM in the presence and absence of PMT.

Materials and methods

Study design and sampling strategy

The sample for the present follow-up study was obtained from a previous study designed to identify the prevalence of peri-implant diseases and potential associated risk factors among partially edentulous individuals rehabilitated with dental implants [30]. In accordance with ethical principles, all participants were informed of their oral health and referred to the Federal University of Minas Gerais (UFMG), Brazil, for free treatment, or instructed to seek dental care elsewhere.

After a 5-year period, a large task force was employed for the recruitment of the 212 initial participants through direct approach, telephone calls, telegrams, emails, and/or text messages. Thus, 80 individuals who were diagnosed with PM at the initial examination (T1—year 2006) were recovered and underwent a new periodontal/peri-implant clinical examination and microbiological collection (T2—year 2011). These individuals were divided into two groups: one with preventive maintenance therapy during the study period (those carrying out regular PMT with dental visits at least once a year (GTP; $n = 39$)) and another one without preventive maintenance therapy (GNTP; $n = 41$). Individuals diagnosed with peri-implant health and peri-implantitis at T1 were not analyzed due to the lower recovery rate and insufficient sample size for analysis. By these means, this study is not a randomized clinical trial but a 5-year follow-up.

The sampling procedure, the inclusion and exclusion criteria, the data collection, the peri-implant, and the periodontal clinical examinations are summarily presented in study flowchart (Fig. 1) and were described in details elsewhere [6].

The present study was approved by the Research Ethics Committee from the UFMG, Brazil (protocol no. 05650203000-10).

Peri-implant clinical examination

The following clinical parameters for four peri-implant sites in each implant were evaluated according to the methodology proposed by Ferreira et al. [30]: suppuration, peri-implant probing depth (PDi), bleeding on probing (BOPi), and plaque index (PLI) around all implants.

Periodontal clinical examination

Also in accordance with Ferreira et al. [30], complete periodontal examinations were performed and included plaque index, periodontal probing depth (PD), clinical attachment level (CAL), and bleeding on periodontal probing (BOP) for four sites in each tooth.

All clinical parameters were measured at dental and peri-implant sites using manual periodontal probe (PCP-UNC 15, Hu-Friedy, Chicago, IL, USA).

Preventive maintenance procedures

During interviews at T2, special attention was given to the occurrence and frequency of periodontal and peri-implant preventive maintenance within the 5 years following T1. Frequency of PMT was determined by self-reported information and confirmed in dental records (GTP group: at least five dental visits during the evaluation period (mean 5.6 ± 0.3 visits); GNTP group: absence of dental visits during the evaluation period). During PMT visits, the following procedures were performed: (1) periodontal and peri-implant status assessment, (2) application of disclosing agents and oral hygiene

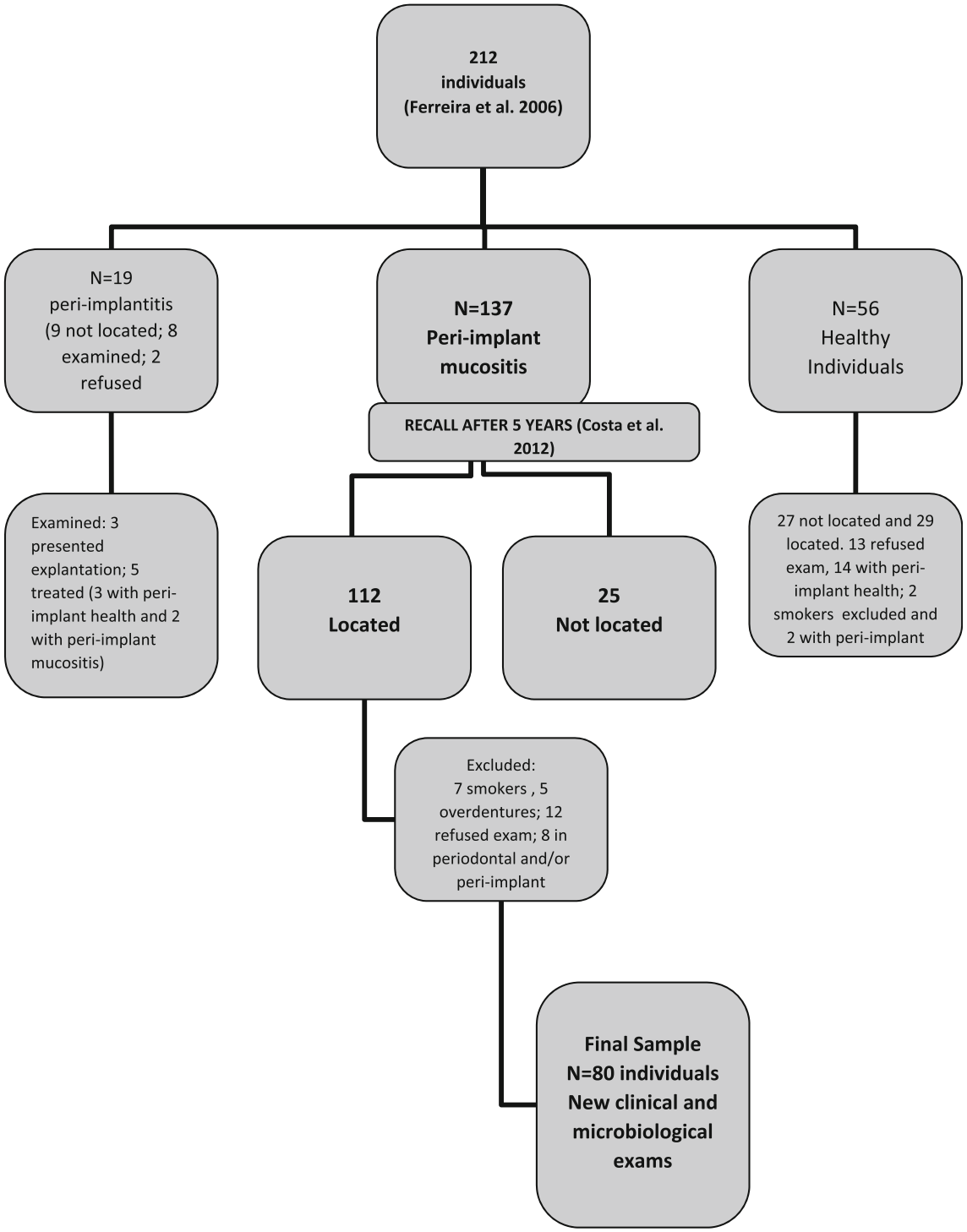

Fig. 1 Participant selection flowchart

instructions, and (3) coronal prophylaxis and non-surgical and surgical mechanical debridement, when necessary.

Diagnostic criteria

Peri-implant mucositis was defined as the presence of visual inflammation and BOPi. Peri-implantitis was defined as the presence of PDi ≥ 5 mm associated with BOPi and/or suppuration with peri-implant bone loss [30]. Cases where the radiographs did not confirm the peri-implant bone loss were diagnosed as PM. It should be noted that these definitions have been re-confirmed and updated according to the recent definition proposed in the recent World Workshop of American Academy of Periodontology and European Federation of Periodontology [1].

Microbiological collection and analyses

Subgingival samples were collected at T1 and T2 in eight peri-implant sites, two in each quadrant (the peri-implant sites with the higher PDi associated with BOPi were evaluated at both times), for each individual as previously reported [31].

Quantification of the total number of bacterial cells, *A. naeslundii*, *P. gingivalis*, *T. forsythia*, *T. denticola*, *P. intermedia*, and *F. nucleatum* was carried out by quantitative real-time polymerase chain reaction (qPCR) using TaqMan assay (TaqMan® Universal PCR Master Mix II, Life Technologies, Carlsbad, USA). The following primers/probes were designed using a primer software (software Primer3 online, Simgene, Hamilton, Canada) and were previously described [32]. *A. naeslundii* (forward: GTCTCAGT TCGGATCGGTGT; reverse: CCGGTACGGCTACC TTGTTA; probe: TACGTTCTCGGGCCTTGTAC), *P. gingivalis* (forward: ACCTTACCCGGGATTGAAATG; reverse: CAACCATGCAGCACCTACATAGAA; probe: VICATGACGATGGTGAAAACCGTCTTCCCTTCTA MRA), *T. forsythia* (forward: AGCGATGGTAGCAA TACCTGTC; reverse: TTCGCCGGGTTATCCCTC; probe: 6FAMCACGGGTGAGTAACGTAMRA), *T. denticola* (forward: CCGAATGTG CTCATTTACATAAAGGT; reverse: GATACCCATCGTTGCCTTGGT; probe: 6FAMATGGGCCCGCGTCCCATTAGC TAMRA), *P. intermedia* (forward: 5′ AAT ACC CGA TGT TGT CCA CA 3′; reverse: 5′ TTA GCC GGT CCT TAT TCG AA 3′; probe: 5′ TGA CGT GGA CCA AAG ATT CAT CGG TGG A 3′), *F. nucleatum* (forward: GCAGCTTCAAATGA TTCGAGTA; reverse: AAGCTTGGTAAAGGCTCTGA AG; probe TTGAAATAAAGAAGAAAAATGGAGG), and universal (forward: TGGAGCATG TGGTTTAATTCGA; reverse: TGCGGGACTTAACCCAACA; probe: VICCACGAG CTGACGACAAGCCATGCATAMRA) in an ABI 7500 Fast Real-Time PCR System® (Life Technologies, Carlsbad, USA) following manufacturer's instructions in 20-μl reactions.

The absolute quantification of the target organism was determined by the plotting of the cycle threshold (Ct) value obtained from each clinical sample against a standard curve generated with a known concentration of gDNA of reference bacterial strains in 10-fold serial dilutions. Negative control (purified PCR-grade water instead of the DNA template) was included in all PCR.

Statistical analysis

A univariate analysis for all comparisons between GNTP and GTP groups was performed using chi-square, Fischer's exact, Mann-Whitney, and Wilcoxon tests, when appropriate.

For the analyses of bacteria counts and bacterial complexes, natural logarithm (exponent) was used due to number size. This logarithm was used to evaluate bacterial frequency (by average and standard deviation) at the two examination times (T1 and T2) in the GTP and GNTP groups. Supplementary to that and following a normal data distribution, marginal linear models were performed to compare the bacterial counts and the bacterial complexes at T1 and T2, between groups and diagnostics (PM and PI), adjusting for the following potential confounders: smoking, diabetes, and plaque index.

The outliers were identified through the standardization of the results, so that the average of the variable was 0 and the standard deviation was 1. For this purpose, observations with standardized scores outside the range of 3.29 were considered outliers.

Statistical analyses were performed using the R software (Windows OS, version 3.2.0), and the results were considered statistically significant if p value < 0.05%.

Results

The characteristics of the sample at T1 and T2 are presented in Table 1. Individuals in the GNTP group had significantly higher values of plaque index when compared to GTP after 5 years (1.9 ± 0.5 vs. 1.4 ± 0.7; $p = 0.001$). Additional data on the periodontal/peri-implant clinical parameters in relation to variables of interest were previously reported by Costa et al. [6].

There was a significantly higher incidence of PI in GNTP (43.9%) than in GTP (18%) group. It is noteworthy that patients with PI in GTP, despite maintenance and necessary surgical treatment, still persisted with PI diagnosis in the final exam. All subjects ($n = 12$) who presented PM resolution at T2 were in the GTP group. There was an increase in the number of individuals with PE in GNTP when comparing T1 (22.0%) with T2 (41.5%) (Table 1).

Table 2 reports intra-group comparisons between T1 and T2 of the total bacterial load (TBL) and the isolated frequency of each pathogen, the frequency of the red complex (*T. forsythia*, *P. gingivalis*, and *T. denticola*), and the frequency of the two bacteria evaluated in the orange complex (sum of the counts of *P. intermedia* and *F. nucleatum*) in the unadjusted and adjusted models.

In the GTP group, there was a significant decrease in TBL, in the frequency of the bacteria analyzed in the orange complex, and in the isolated frequency of *T. forsythia*, *P. gingivalis*, *P. intermedia*, and *A. naeslundii* at T2 (unadjusted and adjusted models). Additionally, there was a significant increase in the isolated frequency *F. nucleatum* (unadjusted model) at T2 (Table 2).

In intra-group comparisons, there was a significant increase in TBL, in the frequency of the bacteria analyzed in the orange complex, and in the isolated frequencies of *P. gingivalis*, *P. intermedia*, and *F. nucleatum* in GNTP. There was an

Table 1 Characteristics of the sample at T1 and T2

Variables	Baseline (T1)			Final examination (T2)		
	GNTP $n = 41$	GTP $n = 39$	p	GNTP $n = 41$	GTP $n = 39$	p
Gender[a]						
Male	22 (53.7%)	24 (61.5%)	0.476	22 (53.7%)	24 (61.5%)	0.476
Female	19 (46.3%)	15 (38.5%)		19 (46.3%)	15 (38.5%)	
Age (years)[b]	46.3 ± 10	42.7 ± 13	0.171	51.4 ± 10.5	48 ± 13	0.195
Smokers/former smokers[a]						
Yes	13 (31.7%)	8 (20.5%)	0.255	14 (34.1%)	8 (20.5%)	0.172
No	28 (68.3%)	31 (79.5%)		27 (65.9%)	31 (79.5%)	
Diabetes[a]						
Yes	6 (14.6%)	5 (12.8%)	0.814	6 (14.6%)	7 (17.9%)	0.688
No	35 (85.4%)	34 (87.2%)		35 (85.4%)	32 (82.1%)	
Number of teeth[b]	849	805	0.927	846	797	0.794
	20.6 ± 6.2	20.6 ± 7		20.6 ± 6.2	20.3 ± 6.9	
Average of lost teeth[b]	2.9 ± 3.9	4.3 ± 5.6	0.283	2.9 ± 3.9	3.0 ± 4.8	0.607
Implant number[b]	183	157	0.143	180	156	0.419
	4.4 ± 3.8	3.9 ± 2.1		4.4 ± 3.8	4.5 ± 3.1	
Installation time of the prosthesis (months)[a]	21.3 ± 7.1	24.7 ± 17.4	0.454	80.5 ± 9	77.4 ± 12.5	0.457
Plaque index[b]	1.6 ± 0.6	1.4 ± 0.6	0.176	1.9 ± 0.5	1.4 ± 0.7	*0.001*
Periodontal diagnosis[a]						
Healthy	32 (78.0%)	29 (74.4%)	0.698	24 (58.5%)	28 (71.8%)	0.214
PE	9 (22.0%)	10 (25.6%)		17 (41.5%)	11 (28.2%)	
Peri-implant diagnosis[c]						
Healthy	0	0	NA	0 (0.0%)	12 (100%)	*0.000*
PM	41	39	NA	23 (56.0%)	20 (51.2%)	
PI	0	0	NA	18 (43.9%)	7 (18%)	

n (%)

GNTP no periodontal/peri-implant preventive maintenance group, *GTP* periodontal/peri-implant preventive maintenance group, *PE* periodontitis, *PM* peri-implant mucositis, *PI* peri-implantitis, *NA* not applicable

[a] Chi-square test

[b] Average ± standard deviations compared by Mann-Whitney test

[c] Fisher's exact test

increase in the frequency (unadjusted model) of the red complex and *A. naeslundii* (Table 2).

Inter-group comparisons (adjusted for smoking, diabetes, and plaque index), as well as the changes in peri-implant diagnosis from T1 to T2, are presented in Figs. 2 and 3.

At T2, individuals diagnosed with PM and PI in the GNTP group presented a significantly higher TBL when compared to GTP. In both groups, individuals who progressed from PM to PI showed a significant increase in TBL. The GTP individuals who remained with PM showed a decrease in TBL (Fig. 3).

The analysis of the isolated frequencies of *T. forsythia*, *P. gingivalis*, *T. denticola*, *P. intermedia*, *F. nucleatum*, and *A. naeslundii* is presented in Fig. 2. Comparisons of total bacterial load, red complex, and two representative bacteria of the orange complex are presented in Fig. 3. The results showed that there were no significant differences between groups in the frequency of the red complex and in the isolated frequency of *T. forsythia* (Fig. 3). At T2, individuals with PM and PI in the GNTP group had a significantly higher frequency of *P. gingivalis*. Individuals who progressed from PM to PI showed a significant increase in the frequency of *P. gingivalis* in GNTP. The GTP individuals who remained with PM showed a decrease in the frequency of *P. gingivalis*. In the GTP and GNTP groups, individuals diagnosed with PI showed a higher frequency of *P. gingivalis* when compared to those diagnosed with PM at T2. Individuals with PI in the GNTP group presented a higher frequency of *T. denticola* at T2 when compared to individuals with PM (Fig. 2).

The frequencies of the bacteria analyzed in the orange complex (sum of *P. intermedia* and *F. nucleatum*) (Fig. 3) and *P. intermedia* (Fig. 2) were significantly lower in individuals with PM in the GTP group at T1 and T2, as in individuals with PI at T2. Individuals who remained with PM in GNTP showed

Table 2 Total sample and intra-group comparisons of total bacterial load, isolated frequency of pathogens, and red and orange complexes at T1 and T2

Variables	Total sample					GTP						GNTP					
	T1		T2		p[b]	T1		T2		p unadjusted[b]	p adjusted[c]	T1		T2		p unadjusted[b]	p adjusted[c]
	Average	SD	Average	SD		Average	SD	Average	SD			Average	SD	Average	SD		
ln (TBL)	15.51	1.49	15.50	1.52	0.998	15.45	1.58	15.07	1.55	*0.001*	*0.001*	15.57	1.41	15.91	1.38	*0.001*	*0.029*
ln (red complex)	12.12	2.97	12.04	3.22	0.891	12.01	2.91	11.64	3.10	*0.011*	0.079	12.22	3.06	12.42	3.32	*0.014*	0.917
ln (orange complex[a])	13.76	3.24	13.91	3.56	*0.037*	12.09	2.74	11.81	2.96	*0.024*	*0.013*	15.34	2.88	15.90	2.89	*0.000*	*0.008*
ln (*T. forsythia*)	10.17	3.37	10.04	3.43	0.408	9.97	3.32	9.52	3.31	*0.000*	*0.000*	10.35	3.45	10.54	3.52	*0.010*	0.942
ln (*P. gingivalis*)	6.52	3.62	6.50	4.03	0.640	5.99	2.85	5.26	3.44	*0.003*	*0.001*	7.02	4.21	7.68	4.23	*0.000*	*0.011*
ln (*T. denticola*)	8.75	4.40	8.79	4.39	0.560	8.96	4.38	9.12	4.27	0.620	0.495	8.54	4.47	8.49	4.53	0.622	0.537
ln (*P. intermedia*)	13.69	3.34	13.83	3.67	0.064	11.98	2.90	11.68	3.09	*0.026*	*0.013*	15.32	2.91	15.88	2.94	*0.000*	*0.009*
ln (*F. nucleatum*)	7.07	1.88	7.40	1.78	*0.000*	7.02	1.67	7.16	1.59	*0.000*	0.144	7.11	2.08	7.63	1.94	*0.000*	*0.000*
ln (*A. naeslundii*)	14.27	3.24	14.23	2.81	0.138	14.13	2.94	13.53	2.52	*0.000*	*0.011*	14.39	3.53	14.90	2.93	*0.007*	0.085

Statistically significant values in italics

TBL total bacterial load

[a] Two representative bacteria of the orange complex: *P. intermedia* and *F. nucleatum*

[b] Wilcoxon test

[c] Marginal linear model adjusted for smoking, diabetes, and plaque index

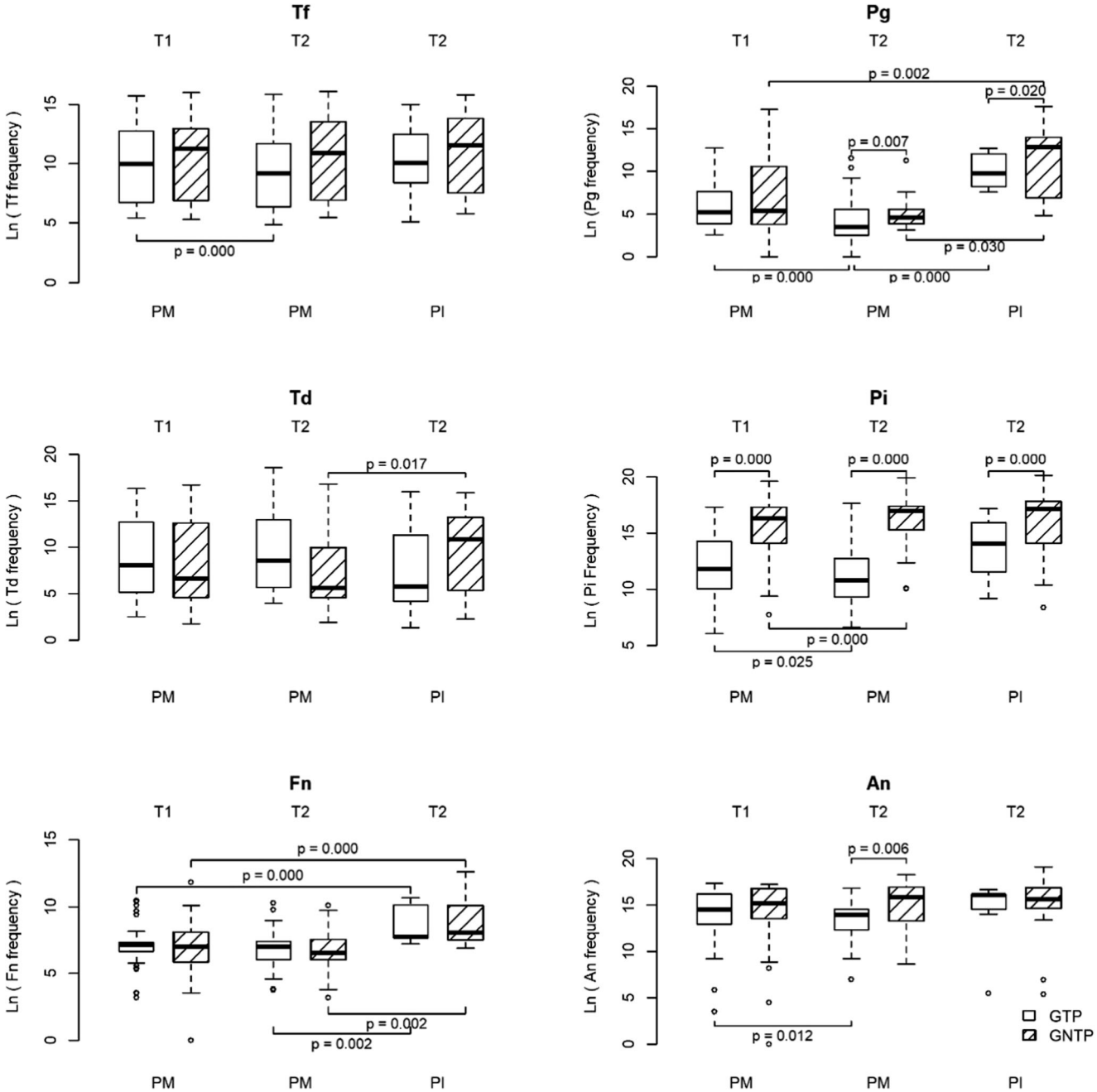

Fig. 2 Comparisons of the frequency of *T. forsythia*, *P. gingivalis*, *T. denticola*, *P. intermedia*, *F. nucleatum*, and *A. naeslundii*: intra- and inter-group, and between diagnosis at each evaluation time, controlling for smoking, diabetes, and plaque index. PM peri-implant mucositis, PI peri-implantitis, GTP periodontal/peri-implant maintenance therapy group, GNTP no periodontal/peri-implant maintenance therapy group (marginal linear models)

a significant increase in the frequency of these bacteria in the orange complex, while in GTP, there was a significant decrease (Fig. 3). *P. intermedia* presented a significantly higher frequency in individuals with PI in GNTP (Fig. 2). The isolated frequency *F. nucleatum* analysis showed that, in both groups, individuals who developed PI showed higher frequencies of these bacteria. At T2, PI individuals in both groups showed an increase in the isolated frequency *F. nucleatum* when compared to individuals with PM (Fig. 2).

Among individuals diagnosed with PM at T2, the frequency of *A. naeslundii* was significantly lower in the GTP group. Individuals with PM at T2 and who performed periodontal/peri-implant maintenance (GTP group) showed a significant decrease in the frequency of *A. naeslundii* (Fig. 2).

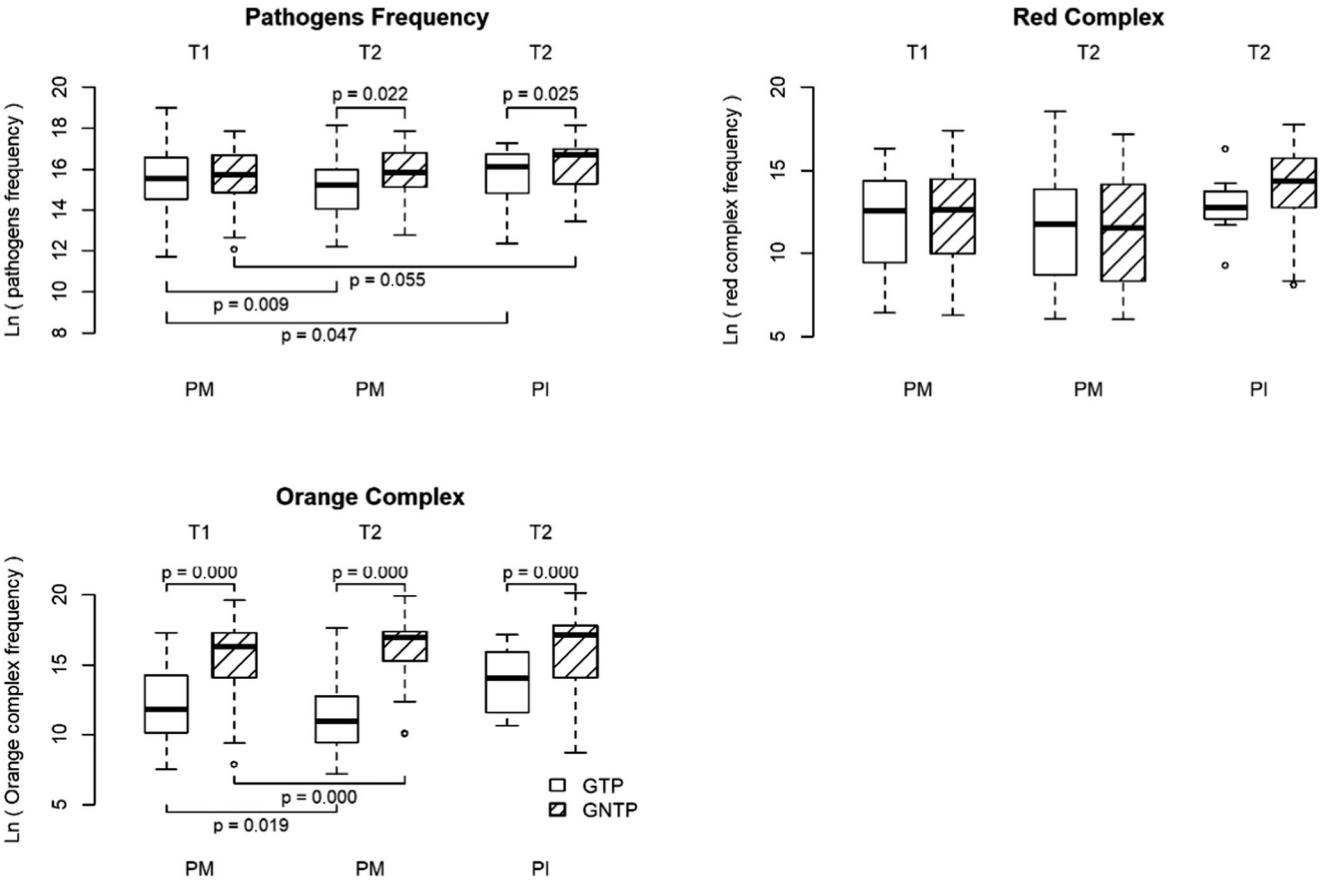

Fig. 3 Comparisons of total bacterial load, red complex, and two representative bacteria of the orange complex: intra- and inter-group, and between diagnosis at each evaluation time, controlling for smoking, diabetes, and plaque index. PM peri-implant mucositis, PI peri-implantitis, GTP periodontal/peri-implant maintenance therapy group, GNTP no periodontal/peri-implant maintenance therapy group (marginal linear models)

Discussion

The results of the present study showed that individuals in the GNTP group presented higher incidence of PI, higher plaque index at T2, higher TBL, and higher counts of the majority of the evaluated bacteria. These findings should be highlighted and corroborate previous studies signaling that higher plaque index and absence of PMT [4, 21, 29, 33], and consequently a greater presence of pathogens for extended periods could collaborate for the increased incidence of PI [27, 34, 35]. Thus, the higher TBL and plaque index in GNTP can hypothetically suggest that the increase in the number of pathogens that could migrate to peri-implant sites can be a risk factor for the development of PI [13–15, 17].

Specifically, the increased frequency of the two pathogens evaluated in the orange complex and also some isolated species (*P. gingivalis*, *P. intermedia*, and *F. nucleatum*) in GNTP, and the reduction in TBL and in the frequencies of *T. forsythia*, *P. gingivalis*, and *P. intermedia*, in GTP, suggested that the absence of PMT can impact on the amount of pathogens found around implants. An important aspect of these findings also suggest that, in the present study sample, the quantitative characteristics of peri-implant microbiota appear to be more important than qualitative characteristics, confirming the results from previous studies [3, 25, 36]. However, surprising findings were also observed as a significant increase in the isolated frequency of *F. nucleatum* and a decrease in *A. naeslundii* in the GTP group.

The fact that all individuals who presented remission of PM were in the group that performed PMT also reinforces the importance of routine visits to peri-implant disease control, especially PM, since it is regarded as a pathological condition that is reversible only with clinical treatment [6, 37]. Additionally, as reported by Salvi et al. [10], PM may require a strict plaque control over 3 weeks for returning to levels of gingival and peri-implant mucosal health.

Results from the present study confirm data from the literature on the importance of regular PMT visits in the control of peri-implant diseases [7, 9, 37, 38]. The absence of PMT was also associated with increased risk of developing PI in recent systematic reviews [4, 37, 38].

Microbiological results from the present study were adjusted for potential confounders for periodontal and peri-implant diseases, such as smoking, diabetes, and plaque index. These confounding variables can, by themselves, affect the microbiological findings [12]. However, comparisons with other findings are limited because few studies have assessed these associations on dental implants [19, 29, 33].

Differences in TBL and in the isolated frequency of some pathogens were reported when comparing individuals diagnosed with PM and PI. Individuals who had a poorer peri-implant condition over time presented more TBL in both groups (GTP and GNTP), and also an increase in the frequency of *P. gingivalis* and *F. nucleatum*, supported by previous findings [19]. Conflicting results were reported in a study where levels of *P. gingivalis* and *F. nucleatum* were not associated with the presence of PI [28]. Findings from the present study showed no significant increase in the red complex bacteria among individuals who progressed to PI compared to individuals with PM. Nevertheless, when the pathogens were analyzed isolatedly, a higher frequency of *P. gingivalis*, *T. denticola*, and *F. nucleatum* was found in individuals with PI at T2. Similar results were described in studies with short periods of monitoring [13, 29, 39, 40].

While bacteria of the red and orange complexes are often associated with the presence of PE and the increase of probing depth, the blue complex has not been related to the presence of periodontal disease [11]. Healthy implants usually have a microbiota consisting of gram-positive bacteria, bacilli, and coccus [14, 34, 39, 41]. However, Renvert et al. [19] found no differences between the microbiota of teeth compared to healthy implants.

Recently, the steps of biofilm formation were reviewed [42]. *Actinomyces* species and the oral *Streptococci* are the primary bacteria to stick in the acquired film and interact with each other by favoring the colonization of both secondary colonizers as *F. nucleatum* and the red complex. Therefore, as the biofilm increases in thickness or in quantity, there is a gradual growth that later becomes constant for several species, including primary colonizers [43]. In teeth, for example, *Actinomyces* species may be most prevalent in both the supra and subgingival biofilm and both in health and periodontal disease [13, 44]. This could hypothetically explain the reduction of the *A. naeslundii* and increase of the *F. nucleatum* in the GTP group. However, there are great challenges in the interpretation of studies on subgingival biofilm in a multifactorial manner. Nevertheless, it is important to note that any change in the environment can have an impact on the microbiota (increase and/or reduction of species), which in turn is capable of inducing changes in the host's response, generating an amplification loop of the periodontal disease process [45].

Hence, the present study showed that after 5 years, there was a trend of increased frequency of pathogens in implants that progressed to PI and in individuals with PM who were not regular in PMT. Despite that, there are great challenges in the interpretation of studies on subgingival biofilm in a multifactorial manner [45]. Therefore, PMT can be an important tool to generate positive impacts on the subgingival microbiota.

To the best of our knowledge, this is the first study on the frequency of different pathogens in the presence or absence of PMT in individuals diagnosed with PM accompanied during 5 years, which can be considered a long monitoring period. Few longitudinal studies on monitoring bacterial profile are found in the literature, and most monitoring periods and samples are short [10, 14, 15, 46–49].

The present study has the limitation of evaluating few bacterial species that are commonly associated with PE and related to PI. In this sense, other PI-related pathogens may have been overlooked, since there are reports of other bacterial species that are not commonly listed in the pathogenesis of PE, but were found in sites with PI [24]. However, the peri-implantitis microbiome is commensal-depleted and pathogen-enriched, harboring traditional and new pathogens. The core peri-implant microbiome harbors taxa from genera often associated with periodontal inflammation [48, 49]. Thus, additional clinical and microbiological longitudinal studies, since the installation of the implant, are required in order to verify the probable bacterial succession that occurs in implants that are subject to the development of PI and its association with different risk factors.

Conclusions

It might be concluded that there was a significant longitudinal increase in TBL, in the frequencies of *P. gingivalis*, *P. intermedia*, and the two pathogens evaluated in the orange complex in GNTP. On the other hand, these same pathogens and *T. forsythia* showed a reduction in GTP. Additionally, individuals who progressed from PM to PI showed significantly higher TBL and frequencies of *P. gingivalis*, *T. denticola*, and *F. nucleatum*. Consequently, it was observed a beneficial role of PMT in maintaining peri-implant clinical stability and homeostasis of the microbiological condition.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in the present study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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