Antibiotics plus probiotic as an adjunct to the treatment of periodontitis in smokers: a short-term study

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Abstract

Aim: The purpose of this study was to perform a short-term evaluation of the effects of a probiotic, associated with the use of antibiotics as an adjuvant treatment of periodontitis in smokers.

Materials and Methods: Thirty-four patients were randomly divided into: PRO group (n = 17), patients received a single session of scaling and root planing (SRP) associated with the systemic administration of *Lactobacillus reuteri* tablets; ANT+PRO group (n=17), patients received a single session of SRP followed by the systemic administration of Amoxicillin and Metronidazole 3x/day for 7 days and administration of *Lactobacillus reuteri* seven days after conclusion of antibiotic therapy. Clinical periodontal parameters were evaluated.

Results: After treatment, both groups showed a reduction in BOP, PI, and residual pockets compared to baseline, while in the ANT+PRO group there was a significant reduction in PD and CAL gain ($p<0.05$). A significant reduction in the number of pockets with PD>5mm was observed in the ANT+PRO group ($p<0.05$).

Conclusion: Both adjuvant treatments reduced the residual pockets and controlled inflammation after treatment. Only the association of antibiotic with probiotic therapy was able to efficiently reduce mean PD, the number of pockets with PD >5 mm, and promote additional CAL gains in a short-term evaluation period.

Keywords: Periodontitis; Antibiotics; Smoking; Periodontal treatment.

Introduction

Periodontitis is a chronic multifactorial inflammatory disease characterized by the inflammation continuum and dysbiosis between the microbiome and host immunologic response, leading to a progressive destruction of tissues surrounding the tooth (Caton *et al*., 2018; Van Dyke *et al*., 2020). Periodontal diseases are quite common, affecting approximately 20-50% of the global population (Benjamin, 2010), and are considered one of the main causes of tooth loss.

Environmental risk factors like smoking cause alterations in the immune and inflammatory system and have a negative impact on several systemic inflammatory diseases, as well as on periodontitis (Jepsen *et al*., 2018). It is well-known that smokers have a high prevalence and severity of periodontitis, exhibiting higher scores of clinical attachment loss (CAL), probing depth (PD), gingival recession (GR), less bleeding on probing (BOP), increased alveolar bone loss, and a higher incidence of tooth loss when compared to non-smoking

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patients (Tomar and Asma, 2000; Labriola *et al*., 2005). Indeed, some authors have suggested that smokers are also more prone to colonization by periodontopathogenic microorganisms than non-smokers, whereas others did not find such results (Décaillet *et al*., 2012).

Scaling and root planing (SRP) associated with oral hygiene instruction is considered the gold standard treatment of inflammatory periodontal diseases (Berezow and Darveau, 2011). This therapy aims to remove and disorganize the complex biofilm formed on the dental surface, supra- and subgingivally, to control the microbial periodontal infection (Darby *et al*., 2001; Cobb, 2002). However, especially in deep periodontal pockets, SRP alone may be not able to promote a sufficient symbiosis to achieve and maintain clinical improvements in all subjects over a long period (Socransky and Haffajee, 2002). In addition, clinical studies have reported that smokers respond less favorably to periodontal treatment than non-smokers, pointing to the need for adjuvant therapies to achieve success and longevity of periodontal treatment in those subjects (Pahkla *et al*., 2006; Faveri *et al*., 2014). Thus, aiming to maximize the effects of SRP, several adjuvant therapies have been used to treat and improve the periodontal outcomes in smokers, such as local (Pradeep *et al*., 2013) and systemic (Matarazzo *et al*., 2008) antibiotic therapy, antimicrobial photodynamic therapy (Theodoro *et al*., 2018), and recently, probiotic administration (Theodoro *et al*., 2019).

Probiotics are living microorganisms, which when administered in adequate amounts may bring benefits to the health of the host (Martin-Cabezas *et al*., 2016). They compete with pathogenic bacteria for nutrition and epithelial adhesion, affect systemic and local immunomodulation, produce antimicrobial substances, and improve mucosal barrier function, making probiotics a promising adjuvant therapy to treat periodontitis (Teughels *et al*., 2013). Among the probiotics suggested for the treatment of periodontitis, *Lactobacillus reuteri* is highlighted due to its ability to modulate the immune-inflammatory response, mitigate re-colonization of periodontopathogens and improve the clinical outcomes of periodontal treatment (Tekce *et al*., 2015).

Regarding the treatment of smokers with periodontitis, the use of systemic and/or local antibiotics as adjuvant therapy to SRP has been shown additional clinical benefits compared to SRP monotherapy (Matarazzo *et al*., 2008; Chambrone *et al*., 2016). Furthermore, another clinical trial that compared the effects of the adjuvant use of amoxicillin (AMX) plus metronidazole (MTZ) clinically and microbiologically in smokers and non-smokers with periodontitis showed that both patient groups presented improvements in all clinical parameters 3 months after SRP, with a better outcome in non-smokers (Faveri *et al*., 2014). However, there is no strong evidence available to indicate the use of antibiotic therapy in smokers as an adjuvant treatment for periodontitis (Assem *et al*., 2017). Moreover, few studies have associated antibiotics with probiotics to treat periodontitis (Shah *et al*., 2017).

In view of the benefits provided by the use of antibiotic therapy (Teughels *et al*., 2020) and probiotics in the treatment of periodontitis in systemically healthy patients (Vivekananda *et al*., 2010), and the greater severity of periodontal disease in smokers (Theodoro *et al*., 2018), the authors hypothesized that SRP associated with systemic antibiotic therapy with AMX and MTZ in addition to probiotics therapy would reduce probing depth and improve the clinical attachment level in smokers. Thus, the aim of the present study was to evaluate the clinical outcomes of the association of probiotic therapy with antibiotic therapy as adjuvant therapy in the treatment of periodontitis in smokers through a randomised controlled clinical trial with a short evaluation period.

Materials and Methods

This present study was conducted from July 2017 to March 2019, following the norms of the consort statement for randomised trials (Moher *et al*., 2012). It received approval from the Human Research Ethics Committee of the School of Dentistry of Aracatuba (CAAE: 65069717.8.0000.5420) with Universal Trial Number (UTN) U1111-1235-9776 and Register Number: RBR-55bc85. A parallel randomised controlled clinical study was carried out with a follow-up of 90 days. Prior to the screening and treatment procedure, all subjects were individually informed about the nature of the study and signed a free and informed consent form approved by the Human Ethics and Research Committee.

Sample calculation

The ideal sample size to assure adequate power (80%) was calculated considering a difference of at least 1 mm for clinical attachment level and a standard deviation of 0.94 mm between the groups (Silva *et al*., 2011; Ince *et al*., 2015) with an initial probing depth ≥ 6 mm. Hence, it was determined that a minimum of 14 patients per group would be required to obtain a study power of 80% with a significance level of 5%. Considering the possibility of losing 20% of patients, 17 patients were included in each group.

Patient selection

Thirty-four patients with periodontitis Stage II or III in Grade C (Papapanou *et al*., 2018) were enrolled from the clinic of the Dentistry School of Araçatuba (FOA-UNESP). The patients were added to the study according to the following inclusion criteria: patients who

smoked more than 10 cigarettes/day for at least 5 years, classified as grade C (Papapanou *et al*., 2018); aged between 30 and 70 years; a diagnosis of stage II periodontitis, defined as presenting an interdental clinical attachment loss of 3-4 mm at least, radiographic bone loss extending to coronal third of root; or periodontitis stage III, defined as presenting an interdental clinical attachment loss ≥5 mm and radiographic bone loss extending to mild-third of root (Papapanou *et al*., 2018); and finally a minimum dentition of 15 teeth, excluding third molars. The exclusion criteria were: medical disorders that require antibiotic prophylaxis or which may influence treatment response; a positive history of medications consumption that may affect the periodontal tissues in the previous six months; periapical alterations in qualified teeth; having received periodontal treatment in the previous six months; pregnant or breastfeeding women; carriers of extensive prosthetic rehabilitation; currently undergoing orthodontic treatment; a positive history of metabolic disorders like diabetes, or diseases that may interfere with the inflammatory process or bone metabolism; immune disorders; alcoholism and consumption of illicit drugs. Patients that met the inclusion criteria were included and evaluated clinically.

Primary and secondary outcomes

Full mouth clinical attachment level (CAL), probing depth (PD), bleeding on probing (BOP), plaque index (PI) and gingival recession (GR) were recorded at baseline (pre-treatment) and at 90 days (post-treatment). All clinical exams were performed in six sites per tooth with a periodontal probe (PCPUNC-15, Hu-Friedy, Chicago, IL, USA) by a calibrated examiner blinded to the treatments (MAAN). The primary outcome of the study was CAL gain. Reductions in PD, BOP, PI, GR, and residual pockets were quantified as secondary outcomes.

Examiner calibration

The calibration procedure was conducted in 20 sites per patient. In total, 120 sites (6 patients) with probing depth ≥ 5 mm were randomly selected and analyzed twice and on different days (7-day interval). The data were submitted to the Kappa agreement test ($p \leq 0.05$).

Experimental design and treatment protocol

Baseline measurements were performed prior to the treatment, after which the patients received detailed information about oral hygiene instruction and the etiology of periodontal disease. The 34 patients were divided into two groups using an online randomiser (www.sealedenvelope.com). An independent investigator (LHT) prepared envelopes containing the treatment distribution, which were opened only on the day of treatment after SRP. Participants were allocated randomly to one of the following groups: PRO group (n = 17), patients received a single session of SRP for approximately 2 hours using ultrasonic instruments (ProfiNeo, DabiAtlante, Ribeirão Preto, SP, Brazil) and hand curettes (Gracey curettes, Hu-Friedy, Chicago, IL, USA) associated with the systemic administration of *Lactobacillus reuteri* (DSM 17938, with 1×108 cfu live bacteria of each strain per lozenge; BioGaia™, 450 mg, Laboratórios Ferring Ltda, São Paulo, SP, Brazil) tablets twice a day for 21 days; ANT+PRO group (n=17), the patients were submitted to the same SRP protocol, followed by the systemic administration of AMX (500 mg) and MTZ (400 mg) three times per day for 7 days. In addition, 7 days after the conclusion of the antibiotic therapy, administration of *Lactobacillus reuteri* was prescribed, twice a day for 21 days.

All chewable probiotic tablets, AMX (500 mg), and MTZ (400 mg) were removed from their packaging and placed in identified vials. The vials with probiotics and antibiotics were identical; however, they contained different descriptions on the packaging label to enable only the professional to differentiate them. Two tablets of probiotics were administered daily, after oral hygiene for twenty-one days. All patients were instructed not to use any products containing anti-inflammatories, antibiotics, or probiotics for 90 days (Teughels *et al*., 2013).

The clinical procedures of all groups were performed by two specialists (DMJM and JMMN), different from those who performed the clinical exams (MAAN). The periodontal procedures were always performed under anesthesia.

Follow up

Patients returned one week after SRP for clinical revaluation, to answer a questionnaire, and for assessment of possible side effects of the treatment, such as diarrhea, allergy, headaches and malaise. Patients from both groups returned 7 and 35 days after SRP treatment. Aiming to evaluate adherence to the treatment, patients were asked to return with the vials of tablets so that the remaining tablets could be counted.

Ninety days after initiation of the treatment, the patients were again contacted for a full mouth periodontal clinical evaluation. At this moment, the use of concomitant medication, all adverse effects, and the medical history were recorded.

Statistical analyses

All demographic data and clinical parameters were tabulated and analyzed using statistical software (Bioestat 5.3, Mamiraua Institute, Manaus, AM, Brazil), at a significance level of 5%. For the calibration of the examiner, the data were submitted to the Kappa agreement test, obtaining a value of 0.83, which represents

a significant intra-examiner agreement. The means and standard deviations of the PD, GR, and CAL were calculated for all sites. Categorical BOP and PI data were transformed into percentages, and the means and standard deviations were obtained. The number of residual pockets (sites with PD ≥ 5 mm and bleeding) were also evaluated, as these may represent the efficiency of periodontal therapy (Lang and Tonetti, 2003). All data were submitted to a normality analysis using the Shapiro-Wilk test. Intra- and intergroup analyses were performed for all periodontal clinical parameters in the two evaluated periods. In the intragroup analysis, the parametric data were submitted to the paired T-test, and the non-parametric data to the Wilcoxon test, comparing baseline with 90 days in both groups. In the intergroup analysis, independent T-tests were performed for the parametric data and the Mann-Whitney test for the non-parametric data, both comparing the groups in the given period. Fisher's exact test was used to compare the differences between sexes.

Results

In total, 167 patients were evaluated, of which 34 were included and treated in this study: 24 men and 10 women, with a mean age of 49.16 years (32-62 years). There were no differences in sex and age between the groups. The average number of cigarettes smoked per day was 19.07 (±10.48) in the PRO group and 17.33 (±8.57) in the ANT+PRO group (Table 1). Twenty-eight patients were also evaluated 90 days after SRP (17.65% drop out), with 14 patients remaining in each group throughout the follow-up period. Three patients in each group were excluded from the study due to non-attendance at the 90 days re-evaluation (4) or the use of anti-inflammatory drugs (2) (Figure 1).

Adverse effects

No patients reported side effects of the medications in either group. However, 2 male patients in the ANT + PRO group reported an improvement in bowel function.

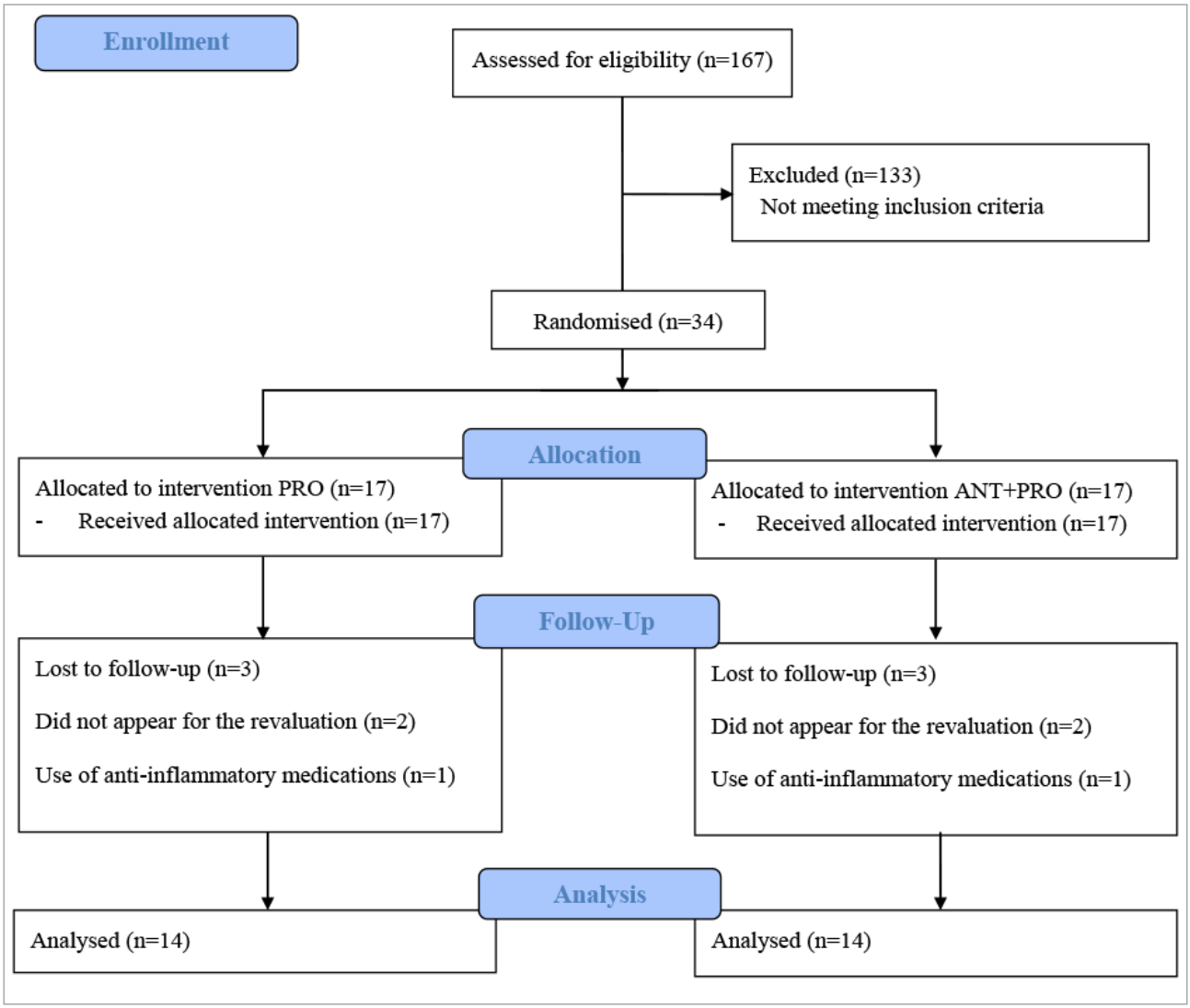

Figure 1. Flow chart of the study.

Clinical findings

In the intragroup analysis of the clinical parameters of the full mouth, a significant improvement in PD and CAL was observed only in the ANT+PRO group (p=0.043; p=0.009). Reductions in BOP, PI and residual pockets (PD ≥ 5 mm, and BOP) were observed in both groups 90 days post-treatment (p <0.05; Table 1). In the intergroup analysis, PI (p=0.0001), GR (p=0.028), pockets with PD ≥ 5mm and BOP (p=0.03) differed between the groups at baseline. Only PI (p=0.0002) and GR (p=0.0004) showed a significant difference between the groups 90 days after the treatment (Table 1).

Table 2 depicts the stratification data of the periodontal pockets (PD ≥ 4 mm and ≤5 mm; PD >5 mm). Both groups showed an improvement in the mean PD and CAL of pockets with PD ≥ 4 mm and ≤5 mm and those with PD≥5mm, as well as in the number of pockets with PD ≥ 4mm and ≤5mm (p<0.05; Table 2). However, in the analysis of the reduction in the number of pockets with PD >5 mm, only the ANT+PRO group presented a significant reduction (p=0.007; Table 2). Additionally, in the intergroup analyses the ANT+PRO group presented significant reduction in the mean PD of pockets with PD >5mm (p=0.036; Table 2). Moreover, 85.71% and 57.14% of patients from the ANT+PRO and PRO group showed ≤4 periodontal pockets with PD ≥5 mm, respectively.

Discussion

This study aimed to evaluate the clinical effects of AMX plus MTZ associated with *Lactobacillus reuteri* as an adjuvant therapy to initial periodontal treatment in smokers. To the best of the author's knowledge, this is the first study to evaluate the adjuvant effects of antibiotic and probiotic supplementation in the treatment of smokers with periodontitis. It was shown that both protocols for adjuvant therapies associated with SRP applied for the treatment of periodontitis in smokers promoted clinical benefits with reductions in mean PD and a CAL gains in pockets with PD ≤ 4 mm and ≤ 5mm, and pockets with a PD >5 mm.

The current study has demonstrated a significant improvement in PD and CAL only in the ANT+PRO group. When the number of pockets with PD >5mm was evaluated, the ANT+PRO group had a significant reduction, and presented in the intergroup analyses a reduction in mean PD of pockets with PD >5 mm. These data suggest additional clinical benefits of the association of antibiotics and probiotics for the treatment of periodontitis in smokers.

Table 1. Demographic characteristics, mean and standard deviation (M ± SD) of the clinical parameters (PD, CAL, GR, BOP, PI, and residual pockets) of the full mouth and number of cigarettes per day at baseline and at 90 days after treatment.

	Groups		
Variable	**PRO (n=14)**	**ANT+PRO (n=14)**	**P value**
Age (years)	46.43 ± 7.36	49.86 ± 7.08	0.57
Gender (M/F)	6/8	12/2	0.59
Nº cigarettes/day	19.07 ± 10.48	17.33 ± 8.57	0.45
PD (mm)			
Baseline	3.22 ± 0.45	3.07 ± 0.46	1.0000
90 days	2.93 ± 0.56	2.67 ± 0.37‖	0.3346
CAL (mm)			
Baseline	4.40 ± 0.85	4.04 ± 0.91	0.2802
90 days	3.92 ± 0.91	3.73 ± 0.85‖	0.3121
GR (mm)			
Baseline	1.16 ± 0.75	1.69 ± 0.87	0.0289
90 days	1.0 ± 0.66	1.9 ± 0.55	0.0004
BOP %			
Baseline	47.75 ± 19.38	38.11 ± 17.09	0.1715
90 days	23.54 ± 14.12‖	18.91 ± 10.24‖	0.3219
PI %			
Baseline	80.98 ± 14.58	53.29 ± 17.99	0.0001
90 days	23.54 ± 14.12‖	34.55 ± 13.02‖	0.0002
Pockets with PD ≥ 5mm and BOP			
Baseline	9.37 ± 7.26	15.87 ± 7.65	0.0307
90 days	3.31 ± 3.04‖	5.22 ± 3.22‖	0.1612

‖ Statistically significant difference between baseline and 90 days in the same group (p<0.05). p valor for PRO vs ANT+PRO. PD: probing depth; CAL: clinical attachment level; GR: gingival recession; BOP: bleeding on probing; PI: plaque index.

Table 2. Evaluation of categorized periodontal pockets.

Clinical parameters	Groups		P value
	PRO (n=14) M ± SD	ANT+PRO (n=14) M ± SD	
Number of sites with PD 4 - 5 mm per patient			
Baseline	33.21 ± 20.26	26.71 ± 9.89	0.5053
90 days	23.64 ± 18.53 ǁ	16.5 ± 8.21 ǁ	0.4347
Reduction 0-90 days (Δ)	9.57 ± 12.01	10.21 ± 8.08	0.7652
PD mean of pockets with PD 4 - 5 mm			
Baseline	4.29 ± 0.10 mm	4.32 ± 0.10 mm	1.0000
90 days	3.62 ± 0.54 mmǁ	3.49 ± 0.39 mmǁ	0.5503
Reduction 0-90 days (Δ)	0.67 ± 0.51 mm	0.83 ± 0.45 mm	0.3739
CAL mean of pockets with PD 4 - 5 mm			
Baseline	4.41 ± 0.12 mm	4.40 ± 0.11 mm	0.7958
90 days	3.98 ± 0.60 mmǁ	4.08 ± 0.48 mmǁ	0.6305
Reduction 0-90 days (Δ)	0.43 ± 0.63 mm	0.32 ± 0.43 mm	0.7477
Number of sites with PD > 5 mm per patient			
Baseline	7.43 ± 6.22	7.43 ± 9.08	0.6295
90 days	5.35 ± 6.48	3.43 ± 5.69 ǁ	0.2148
Reduction 0-90 days (Δ)	2.07 ± 3.51	4.0 ± 4.57	0.2603
PD mean of pockets with PD >5mm			
Baseline	6.82 ± 0.78 mm	6.64 ± 0.59 mm	0.4950
90 days	5.15± 1.34 mmǁ	4.22 ± 1.10 mmǁ	0.0366
Reduction 0-90 days (Δ)	1.67 ± 1.71 mm	2.42 ± 1.13mm	0.1681
CAL mean of pockets with PD >5mm			
Baseline	7.16 ± 0.65 mm	7.07 ± 0.50 mm	0.8542
90 days	6.14 ± 1.55 mmǁ	5.60 ± 0.89 mmǁ	0.8362
Reduction 0-90 days (Δ)	1.02 ± 1.09 mm	1.47 ± 0.82 mm	0.4347

ǁ Statistically significant difference between baseline and 90 days in the same group ($p<0.05$).
p valor for PRO vs ANT+PRO. M: Mean; SD: Standard deviation.

In the full mouth analysis, a CAL gain was verified only in the ANT+PRO group 90 days after the treatment. The mean CAL gain in pockets between 4-5 mm was 0.43 ± 0.63 mm in the PRO group and 0.32 ± 0.43 mm in the ANT+PRO group. In addition, the mean CAL gain in periodontal pockets with PD > 5 mm was 1.02 ± 1.09 mm in the PRO group, and 1.47 ± 0.82 mm in the ANT+PRO group.

Recently, our research team has shown that the use of *Lactobacillus reuteri* associated with SRP caused a BOP reduction in residual pockets and reduced the mean depth of deep pockets 90 days post-treatment (Theodoro *et al*., 2019). Furthermore, a PD reduction of 3.81 ±0.44 mm to 3.66 ±0.36 mm in the SRP group, and 3.23 ±0.44 mm to 2.98 ±0.54 mm in the PRO group was verified. These results corroborate with another systematic review and meta-analysis that seem to support the adjuvant use of *Lactobacillus reuteri* to SRP in chronic periodontitis treatment in the short term, especially in deep pockets (Martin-Cabezas *et al*., 2016).

The protocol of association between AMX and MTZ has been shown in the literature to be the most relevant, in terms of magnitude and the significance of effects (Teughels *et al*., 2020). Previous studies that evaluated the use of systemic antibiotic therapy (AMX + MTZ) in smokers have also found clinical benefits in moderate and deep pockets after the treatment, when compared with SRP monotherapy (Matarazzo *et al*., 2008; Theodoro *et al*., 2018).

Thus, due to the additional benefits of both adjuvant therapies separately, the present study proposes the combination of these adjuvant therapies in the treatment of smokers with periodontitis.

This improvement in clinical parameters may be associated with the hypothesis that the proposed concomitant use of antibiotics and a probiotic aims to reduce periodontopathogens with antibiotics use, as well as recolonising the oral microbiota with beneficial microorganisms through the use of probiotic (Teughels *et al*., 2013). However, more clinical trials with microbiological analyses are needed to validate this hypothesis.

The greater reduction in pockets with PD>5mm found in this study is in line with previous studies (Cobb, 2002; Theodoro *et al*., 2019). The effect of AMX+ MTZ has been demonstrated in previous studies in systemically healthy patients and in smokers (Theodoro *et al*., 2017;

Theodoro *et al*., 2018). Smoking represents an important modifying factor for the development and progression of periodontitis (Jepsen *et al*., 2018), causing severe insertion and bone losses, especially in the upper molars (Ramesh *et al*., 2019). In addition, immune-inflammatory system changes found in smokers lead to a less favorable treatment outcome after non-surgical periodontal therapy (Johnson and Guthmiller, 2007; Coretti *et al*., 2017; Assem *et al*., 2017).

To evaluate the effectiveness of the periodontal treatment associated with adjuvant therapies, the clinical endpoint was set at ≤4 sites with PD ≥5 mm (Feres *et al*., 2020). In the present study it was identified that 85.71% and 57.14% of patients from the ANT+PRO and PRO group presented ≤4 periodontal pockets with a PD ≥5 mm, respectively.

BOP levels reduced in both groups. BOP is altered in smoking patients, resulting in a strong and chronic suppression of gingival bleeding, a known cigarette-related effect (Dietrich *et al*., 2004). The average of cigarettes smoked on day per patient was 19.07 ± 10.48 and 17.33 ± 8.57 in the PRO and ANT+ PRO group respectively, demonstrating a heavy smoking patient profile in both groups. After treatment, both groups presented a reduction in BOP with 23.54 ± 14.12% and 18.91 ± 10.24% of the bleeding sites in groups PRO and ANT+PRO, respectively. These numbers represent a lower risk of periodontitis progression in the ANT+ PRO group, leaving the endpoint at 1-2 years, since the presence of > 10% and > 20% sites with BOP after treatment increases the risk of reactivated periodontitis in patients (Feres *et al*., 2020).

These data correspond with a previous study that evaluated the adjuvant use of *Lactobacillus reuteri* with SRP in smokers (Theodoro *et al*., 2019). Contrarily, similar results were not confirmed in studies that evaluated the association of AMX and MTZ as an adjuvant therapy to SRP in smokers (Theodoro *et al*., 2018). This fact may be associated with an improvement in the immune system provided by the association of antibiotics with *Lactobacillus reuteri* (Warnakulasuriya *et al*., 2010). Moreover, probiotics may alter the interaction of gingival epithelial cells with *Porphyromonas gingivalis*, modulating the ability of the pathogen to adhere and invade the tissue (Albuquerque-Souza *et al*., 2019), re-establishing the symbiosis in the local environment, and thus, preventing future invasions of periodontopathogens and reducing gingival inflammation (Ikram *et al*., 2019). However, further investigations are still needed to confirm these assumptions.

It is worth pointing out that an important periodontal clinical variable used to evaluate the success of the periodontal treatment is the reduction in pockets with PD ≥5 mm with BOP, also known as residual pockets (Mombelli *et al*., 2015). The fact that the use of *Lactobacillus reuteri* or the association of antibiotic therapy plus *Lactobacillus reuteri* as an adjuvant to SRP promoted this clinical benefit in both groups emphasizes that the proposed therapy was capable of prolonging the effect of periodontal treatment in smokers with periodontitis, reducing the need for further interventions and surgical treatment.

It is worth mentioning that patients' adherence to treatment and oral hygiene enables better results in the short term. In this study, both groups presented a significant PI improvement. After 90 days of treatment, both groups had a PI lower than 40%, which is considered tolerable for the maintenance of periodontal health (Lang and Tonetti, 2003). There was a higher PI reduction in group PRO (23%) than in ANT+PRO (34%) in the present study after 90 days. In line with these results, the literature has shown PI reductions in non-smoking patients with periodontitis following the association of SRP and probiotics therapy with *Lactobacillus reuteri* (Vivekananda *et al*., 2010; Teughels *et al*., 2013). Moreover, other authors have also noted a *Porphyromonas gingivalis* reduction in the SRP + probiotic group, demonstrating positive effects of *Lactobacillus reuteri* therapy on the microbiota in the periodontal pocket. However, a smoker's profile is usually associated with incomplete adherence, demonstrating worse oral health than non-smoking patients (Manicone *et al*., 2017). Therefore, the association of supporting therapies is necessary to decrease the need for future surgical procedures in the treatment of periodontitis in smokers.

Although all 34 patients included in this study were diagnosed with periodontitis Stage II or III, Grade C, a statistical difference was verified between the groups at baseline in GR, PI and in number of pockets ≥ 5 mm and with BOP. These differences at the baseline happened coincidentally and could be a limitation of the allocation process in the randomised clinical trial.

Among the limitations of this study, only clinical parameters where accessed in a short- term follow-up (90 days). Additionally, it can be pointed out that our sample size may be too small to detect the real differences between the groups, and limited additional groups such as SRP and/or antibiotics alone. Further studies with an increased sample size and longer follow up periods are suggested in order to validate the benefits of this therapy in the long term. Indeed, we did not evaluate the effects of both therapies at a microbiological and immunological level to provide scientific proof of their efficacy.

Clinical study of the treatment with probiotic presented a significant reduction of the quantification of periodontopathogens such as *Tanerella forsythia, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum* and *Prevotella intermedia*, after 12 weeks (Teughels *et al*., 2013). In addition,

Vivekananda, *et al*. (2010), showed a reduction in the quantity of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*, at the end of the 3-week intake of the probiotic in patients who took probiotics (*Lactobacillus reuteri*), regardless of whether they were treated or not. However, more clinical trials with microbiological analyses are needed to validate this hypothesis. Thus, further studies are required to determine the advantages of the use of antibiotics associated with probiotics in the treatment of periodontitis in smokers. In addition, additional clinical trials are also needed to clarify the effects of different protocols of antibiotics associated with probiotics as adjuvant therapy in the treatment of periodontitis in smokers.

Conclusion

Within the limits of this study, it can be concluded that both adjuvant treatments were efficient in reducing the residual pockets and controlling inflammation in smokers with periodontitis. However, only the association of antibiotic and probiotic therapy was able to efficiently reduce mean PD and the number of pockets with PD >5 mm, and promote additional CAL gains in a short-term evaluation period.

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