Clinical and microbiological evaluation of the systemic use of doxycycline as an adjunct to basic periodontal therapy in the treatment of individuals with type 2 diabetes and chronic periodontal disease

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Introduction: Doxycycline is a broad spectrum antibiotic derived from tetracycline, which is effective against most periodontal pathogens, by achieving concentrations in the gingival crevicular fluid 7 to 10 times greater than serum level, reducing the signs and symptoms of local periodontal disease. **Objectives:** The aim of this study was to determine the clinical parameters, as well as to quantify the G+ and G- microorganisms in patients with Diabetes mellitus type 2 undergoing basic periodontal therapy with or without the use of antimicrobials, after the total period of 180 days. **Methods:** 23 patients were selected and divided into two groups: Group 1 – basic periodontal treatment; Group 2 – basic periodontal treatment + Doxycycline 100 mg orally for 14 days. **Results:** The results showed an improvement in all evaluated clinical periodontal parameters, as well as the levels of glycated hemoglobin in both treated groups, after 180 days. In Group 2, there was a greater improvement in gingival index of glycated hemoglobin, as well as a smaller amount of G- colonies at the end of the experimental period, in relation to Group 1. **Conclusion:** It is suggested that the systemic use of doxycycline associated with basic periodontal therapy showed clinical and microbiological results more beneficial and superior to basic periodontal therapy only, in patients with type 2 diabetes and chronic periodontal disease. **Keywords:** Diabetes Mellitus. Periodontitis. Doxycycline.

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INTRODUCTION

The American Diabetes Association ranked, in 1997, periodontal disease as the sixth complication of diabetes. The latter increases the risk of periodontal disease and affects its severity. Diabetic individuals tend to present higher rates of plaque index, dental calculus, gingival inflammation and probing depth. When compared to healthy individuals, it is common for most diabetic patients to present with xerostomia and more severe tooth caries.[1]

A decisive factor in periodontal disease is biofilm (bacterial plaque), which is worsened by the histopathological and metabolic changes typical of diabetes. Various degrees of inflammation can be found as being relative to unsatisfactory plaque control. Changes in subgingival surroundings, such as increased glucose levels in the gingival crevicular fluid, favor growth of a few species of bacteria. Vascular changes presented by uncontrolled ill individuals have close connection with periodontal disease establishment and development.[2]

Diabetic individuals' saliva and gingival fluid might have increased amounts of sugar, which could partially affect one's biofilm microbiota, thus exerting influence on the development of caries and possibly periodontal disease.[3]

Several mechanisms have been proposed to explain the greater incidence and severity of periodontal disease among diabetic patients, namely: dysfunctional polymorphonuclear leukocytes, vascular changes, affected synthesis of collagen and glycosaminoglycan, uncontrolled production of cytokines and development of advanced glycation final products. Through its receptors, the latter induces manifestation of pro-inflammatory cytokines, such as prostaglandin E2, interleukin-1b (IL1-b), interleukin-6 and tumor necrosis factor-α.[4]

Abrupt periodontal loss and more severe periodontitis are found among individuals with uncontrolled diabetes, compared to individuals with well-controlled blood glucose levels.[1] Periodontal treatment, by reducing periodontal inflammation, might help restore insulin sensitivity, thus improving glycemic control.[5]

Mechanical therapy carried out by means of scaling and root planning is a requirement to control periodontal infection and, in most cases, is enough to restore periodontal health.[6] Nevertheless, a few variables might be present and associated with unsuccessful outcomes in mechanical therapy. Such variables might be relative to failure in eliminating pathogens due to difficulty in having periodontal scraper accessing the base of periodontal pockets, in addition to anatomical variations of the root or systemic factors affecting the host's response. Some periodontal pathogens are able to enter gingival epithelial cells and subepithelial connective tissue and further recolonize the tooth surface after therapy, particularly when in association with insufficient supragingival bacterial plaque control.[7]

Tetracyclines taken orally have good rates of absorption, and doxycycline is better absorbed than tetracycline chlorhydrate. They are bacteriostatic, bacterial protein synthesis inhibitors, have a broad spectrum, are effective against Gram-positives and Gram-negatives, cocci, as well as aerobic and anaerobic bacteria, in addition to being able to interact with dental tissues. Anti-inflammatory properties of tetracyclines have been recently reported. They inhibit matrix metalloproteinases and other collagenolytic enzymes that destruct the collagen, the fundamental structure of periodontium, and might facilitate fibronectin to adhere to root surfaces, thus allowing periodontal ligament regeneration. Additionally, tetracyclines inhibit bone resorption *in vitro*.[8]

The aim of the present study was to assess clinical and microbiological effects of the systemic use of doxycycline as adjunct to basic periodontal therapy in patients with Diabetes mellitus type 2 associated with chronic periodontitis, after a total follow-up period of 180 days.

MATERIAL AND METHODS

Sample description

A total of 23 patients, aged between 30 and 60 years old, with type 2 diabetes mellitus and uncontrolled metabolism was selected. The sample of diabetic patients was selected from the periodontal clinic at *Universidade Estadual do Oeste do Paraná* (UNIOESTE), based on patients seeking periodontal treatment. In order to assess patients' metabolism as being uncontrolled, their glycated hemoglobin examination should be HbA1c > 7%. This is because 6% is considered as the limit for a diabetic patient's condition to be considered well-controlled, and 1% above the limit would decrease the risk of patients being at a state of transient metabolic change.

Criteria for inclusion were as follows: chronic periodontitis ranging from moderate to severe, whether local or generalized; at least six sites with probing depth above 5 mm and clinical attachment loss greater or equal to 4 mm; bleeding on probing and gingival inflammation; no caries and/or prostheses at clinical examination. Teeth should be in normal position and patients should have at least 20 teeth present in the dental arch. Clinical examination was to be carried out on buccal, lingual/palatal, mesial and distal surfaces.

Criteria for exclusion were as follows: use of antibiotics in the last six months or anti-inflammatory drugs three months before the study, pregnant patients, use of birth-control pills or any other type of hormone, smokers, patients who had undergone periodontal treatment in the last 12 months, and patients with controlled diabetes (HbA1c < 7%).

Data collection

Clinical assessment

Initial clinical examination was carried out by one previously calibrated examiner using a #23 Williams probe. The following was determined:

1) Silnees-Löe plaque index.[9]

2) Löe-Silness gingival index.[10]

3) Bleeding on probing: presence or absence of bleeding 30 seconds after probing depth was measured.

4) Probing depth: distance from the bottom of the pocket to the gingival margin in six points: mesiobuccal, buccal, disto-buccal, disto-lingual/palatal, lingual/palatal and mesiolingual/palatal of every single tooth to be examined.

5) Clinical attachment loss: it corresponds to the distance (expressed in mm) from the amelocementary junction to the bottom of the periodontal pocket in every point of each single tooth. Probing depth was also established using the same points.

Laboratory assessment

All patients taking part in the experiment were asked to seek the doctor in charge of following up their condition (diabetes mellitus) and have glycated hemoglobin laboratory assessment conducted at 0, 90 and 180 days.

After initial clinical examination and initial laboratory assessment, patients were randomly divided into two groups, as shown in Table 1.

Microbiological assessment

Subgingival biofilm samples were collected from four deep sites (probing depth > 5 mm and clinical attachment level > 4 mm with bleeding on probing) and four shallow sites (probing depth < 3 mm and clinical attachment level < 2 mm with bleeding on probing) in different non-adjacent teeth in all individuals. Samples were obtained through sterile absorbent paper cones introduced

Table 1: Grouping of patients (n=23) according to proposed treatment.

Group	ASSESSMENT PERIOD		
	Initial	90 days	180 days
Group 1: 12 diabetic patients	Clinical and laboratory examination. Mechanical control (modified Bass technique + dental floss). Basic periodontal therapy.	Clinical and laboratory examination. Adjunct periodontal therapy.	Clinical and laboratory examination. Adjunct periodontal therapy. Microbiological analysis.
Group 2: 11 diabetic patients	Clinical and laboratory examination. Mechanical control (modified Bass technique + dental floss). Basic periodontal therapy + doxycycline 100 mg orally for 14 days	Clinical and laboratory examination. Adjunct periodontal therapy.	Clinical and laboratory examination. Adjunct periodontal therapy. Microbiological analysis.

up to the pocket's total length for 30 seconds. After sample collection, paper cones were placed into previously labeled threaded tubes containing 3 mL of thioglycollate broth. The tubes were sent to the Laboratory of Bacteriology and Clinical Micrology at UNIOESTE (Cascavel, Paraná, Brazil) where they were inoculated in agar medium with 5% defibrinated lamb blood supplemented with hemin and vitamin K1, so as to allow the sample to bear growth of demanding anaerobic bacteria. All tests were carried out in duplicate.

Trays were incubated at 35-37 °C up to 14 days. GasPak™ disposable system was used to this end, which is an incubation container capable of producing hydrogen and carbon dioxide, with a sachet equipped with a catalyst, designed to create a enriched environment containing 10% H_2, 10% CO_2 and 80% N_2. GasPak™ is suitable for anaerobic bacterial isolation and culture growth and allows slow-growing microorganisms to be detected.

After incubation, each tray was assessed and different types of colonies were selected for primary identification by means of detecting differences in the overall appearance of the trays. Morphological recognition of colonies was carried out by means of identifying the following: size, color, shape, raising level, edge, surface, texture, consistency, opacification, agar effect and effect observed on the blood (none, partial hemolysis, complete hemolysis). Subsequently, they were identified according to morphological and staining characteristics by Gram staining.

Statistical analysis

Clinical data obtained were analyzed by means of Shapiro-Wilk test with a view to checking their distribution, which was rendered normal. Thus, analysis of differences within and between groups was carried out by means of one-way ANOVA. In the event of statistically significant differences, Tukey's test was used to determine the difference between groups and/or periods. Significance level adopted was 5%.

Reliability and reproducibility values were determined with a few measurements carried out by a second examiner blinded in regard to tests and results. The examiner conducted analyses of clinical parameters, within the 90-day period, in all patients. Subsequently, interexaminer agreement was assessed by Kappa statistics. Variables determination, as described, proved to be reliable, since Kappa statistics was equal to 0.836.

RESULTS

Table 2 shows percentage mean values of bleeding on probing, as well as plaque and gingival index at the three assessment periods (0, 90 and 180 days) in all groups, treated as shown in Table 1.

Both groups 1 and 2 showed a decrease in bleeding on probing after 180 days. In group 1, the initial percentage mean value of 39.8 ± 13.7 decreased to 29.7 ± 5.3 on the 180th day, with a decrease of 10.1% in this period ($p < 0.05$). In group 2, the initial percentage mean value of 31.4 ± 16.3 decreased to 18.1 ± 11.8 on the 180th day, with a decrease of 42.4% in this period. Intergroup comparison revealed homogeneity at the onset of the assessment period. As for the second half of the assessment period, on the 90th day, groups revealed statistical similarity. After 180 days, group 2 (18.1 ± 11.8) presented a lower percentage of gingival bleeding, in comparison to group 1 (29.7 ± 5.3).

As for plaque index, there was a decrease after 180 days in both groups. In group 1, the initial percentage mean value of 39.8 ± 9.6 increased to 44.6 ± 7.9 on the 90th day, and then decreased to 33.4 ± 4.0 after 180 days, with a total decrease of 6.4% by the end of the 180-day period. Results were similar in group 2, in which there was an increase from 41.0 ± 25.4 at onset to 45.4 ± 18.2 on the 90th day, and then a decrease to 39.0 ± 17.4 after 180 days, with a total decrease of 2.0% over 180 days. However, there was no statically significant difference between groups in all periods of assessment.

Gingival index also decreased after 180 days of assessment in both groups. In group 1, the percentage mean value of 16.8 ± 3.3 at onset decreased to 15.4 ± 3.7 after 180 days, with a total decrease of 1.4% within this period. As for group 2, the percentage mean value of 11.1 ± 7.8 at onset decreased to 7.4 ± 2.5 after 180 days, with a total decrease of 3.7% within this period. Intergroup comparison revealed differences at onset, which remained after 90 and 180 days.

Table 3 shows percentage mean values of probing depth, as well as clinical attachment loss at the assessment periods (0, 90 and 180 days) in all groups treated as shown in Table 1.

Probing depth had a decrease in both groups when onset and 90-day periods were compared (from 2.7 ± 0.8 to 2.6 ± 0.2 in group 1 and from 2.2 ± 0.7 to 1.9 ± 0.6 in group 2), with significant difference only in group 2. However, after 180 days, there was significant decrease in probing depth for group 1 (to 2.2 ± 0.3) and no changes in group 2 (1.9 ± 0.2). Intergroup comparison revealed group 2 presented significant statistical decrease ($p < 0.05$) when this variable was assessed after 90 and 180 days.

Clinical attachment loss decrease was not significant in group 1 neither in group 2 after 90 days (from 3.3 ± 1.4 to 3.2 ± 0.8 in group 1, and from 3.6 ± 1.2 to 3.4 ± 1.3 in group 2). Values remained steady after 180 days in both groups (3.2 ± 0.4 in group 1, and 3.4 ± 1.5 in group 2). Intergroup comparison revealed statistical similarity between groups in the three assessment periods.

Table 4 shows mean values of glycated hemoglobin at the three assessment periods (0, 90 and 180 days) in both groups, treated as shown in Table 1. There was some improvement in both groups by the end of the 180-day period (from 9.9 ± 3.3 to 8.8 ± 2.3 in group 1, and from 9.7 ± 1.6 to 7.8 ± 1.3 in group 2). In group 1, there was a decrease of 1.1 mg/dL when initial and final assessment periods were compared, with a total decrease of 11.1%. In group 2, there was a decrease of 1.9 mg/dL during the same period, with a total decrease of 19.6%.

Table 2: Bleeding, gingival and plaque index. Values are expressed as mean percentage ± standard deviation (ANOVA, Tukey 5%).

Assessment period	BLEEDING		PLAQUE INDEX		GINGIVAL INDEX	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
0	38.9 ± 13.7^{A}	31.4 ± 16.3^{A}	39.8 ± 9.6 ^{A}	41.0±25.4 ^{A}	16.8±3.3^{A}	11.1± 7.8^{B}
90 days	36.2 ± 11.6*^{A}	26.0 ± 12.7$^{\neq A}$	44.6 ± 7.9*^{A}	45.4±18.2*^{A}	16.4± 1.7*^{A}	8.4 ± 4.1*^{B}
180 days	29.7 ± 5.3$^{\neq A}$	18.1 ± 11.8*^{B}	33.4 ± 4.0$^{\neq A}$	39.0±17.4$^{\neq A}$	15.4 ± 3.7$^{\neq A}$	7.4± 2.5$^{\neq B}$

*, ≠ ($p < 0.05$) statistically different data within the same group. Different letter suggest statistically significant difference in intergroup comparison ($p < 0.05$).

Table 3: Probing depth and clinical attachment loss. Values stand for mean value expressed in millimeters ± standard deviation (ANOVA, Tukey 5%).

Assessment period	PROBING DEPTH		CLINICAL ATTACHMENT LOSS	
	Group 1	Group 2	Group 1	Group 2
0	2.7 ± 0.8 ^{A}	2.2 ± 0.7* ^{A}	3.3 ± 1.4^{A}	3.6 ± 1.2^{A}
90 days	2.6 ± 0.2 ^{A}	1.9 ± 0.6^{B}	3.2 ± 0.8 ^{A}	3.4 ± 1.3 ^{A}
180 days	2.2 ± 0.3* ^{A}	1.9 ± 0.2^{B}	3.2 ± 0.4 ^{A}	3.4 ± 1.5 ^{A}

*($p < 0.05$) statistically different data within the same group. Coinciding letter suggest statistical similarity in intergroup comparison ($p < 0.05$).

Table 4: Glycated hemoglobin values. Values are expressed as mean percentage ± standard deviation (ANOVA, Tukey 5%).

Assessment period	GLYCATED HEMOGLOBIN	
	Group 1	Group 2
1st examination (0)	9.9 ± 3.3 ^{A}	9.7 ± 1.6 ^{A}
2nd examination (90 days)	9.0 ± 1.3* ^{A}	8.6 ± 2.4 ^{A}
3rd examination (180 days)	8.8 ± 2.3$^{\neq A}$	7.8 ± 1.3* ^{A}

*, ≠ ($p < 0.05$) statistically different data within the same group. Coinciding letter suggest statistical similarity in intergroup comparison ($p < 0.05$).

Microbiological analysis

Group 1

In group 1, most colonies were large with coloration varying from light brown to deep dark brown. This group in particular had colonies under complete hemolysis. After Gram staining, Gram-negatives were found in greater amount than Gram-positives. Additionally, the presence of spore-forming Gram-negative bacilli was also acknowledged (Figs 1, 2 and 3).

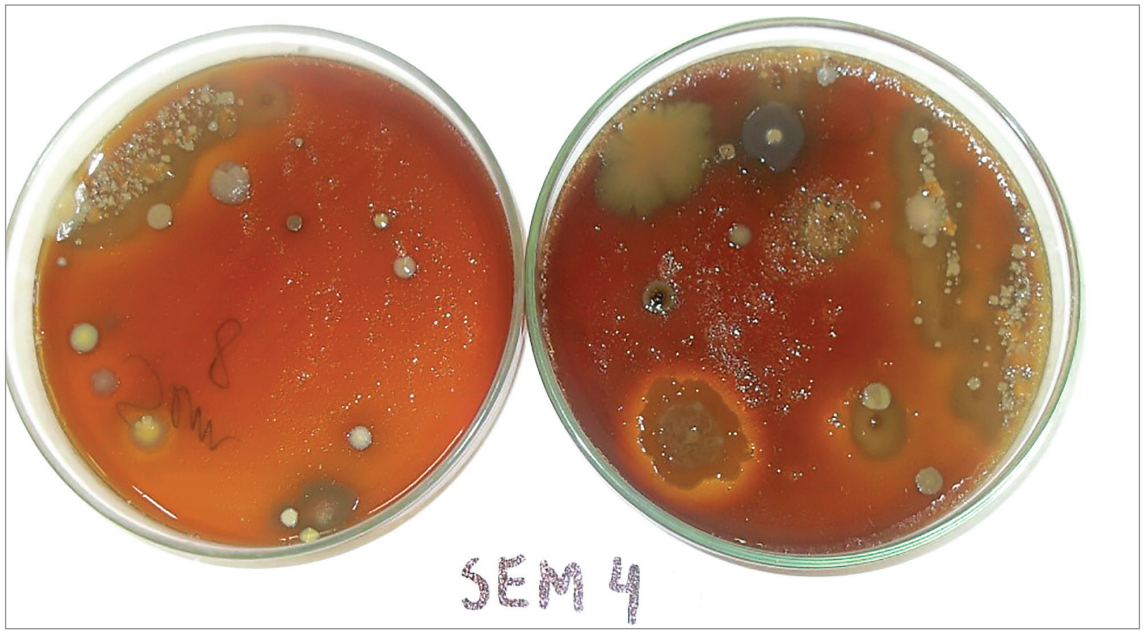
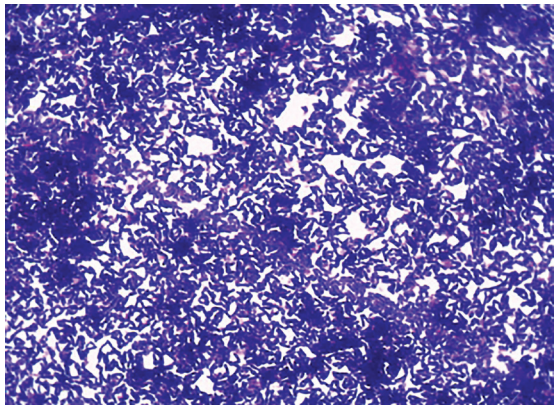
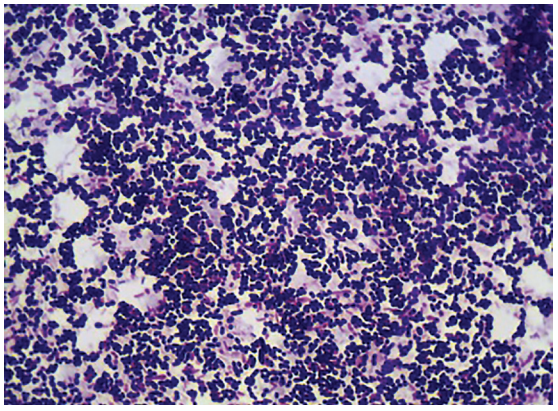


Figure 1: Morphological characteristic of colonies in patient from group 1.





Figures 2 and 3: Morphological and staining characteristics by Gram staining in patient from group 1.

Group 2

In group 2, most colonies coloration was light brown or milky white, presenting no microorganisms under complete hemolysis. In this group, Gram staining revealed a smaller amount of Gram-negative bacilli, a greater amount of Gram-positive cocci and absence of spore-forming bacteria (Figs 4, 5 and 6).

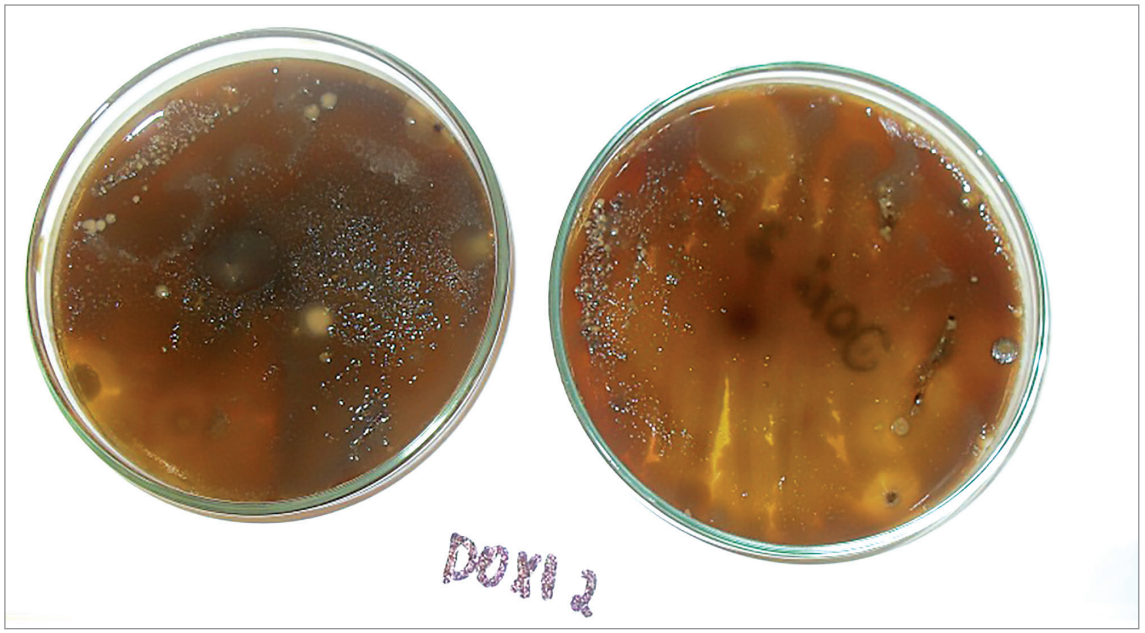
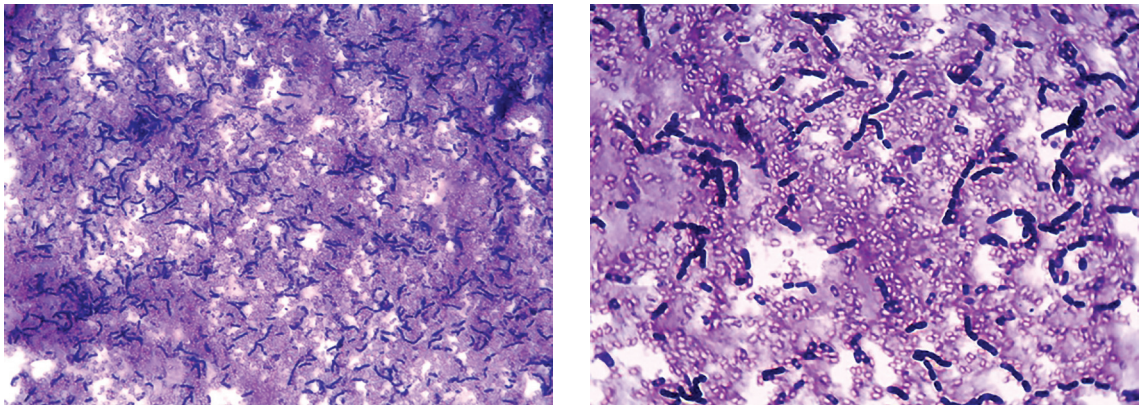


Figure 4: Morphological characteristic of colonies in patient from group 2.



Figures 5 and 6: Morphological and staining characteristics by Gram staining in patient from group 2.

DISCUSSION

Systemic or local use of antimicrobial agents is an alternative to supplement conventional mechanical therapy, with a view to acting against pathogenic microbiota, in addition to affecting the host's inflammatory response, thus restraining tissue loss. Nevertheless, the systemic use of drugs not only reaches dental sites, but also non-dental ones, in addition to acting against bacteria that enter tissues. Additionally, it also has direct and indirect effects on the host's response. On the other hand, this means of drug administration might provide resistance to bacteria, undesirable side effects and low concentrations of medication at the affected site.[11]

In the present study, doxycycline was the antibiotic of choice due to low-dose administration of the drug having proved effective against chronic periodontitis when combined with scaling and root planning.[8,12]

Some authors recommend doxycycline use in low doses for a long period of time in order to have collagenase action inhibited in the host.[12-15] They also stated that low doses of doxycycline combined with scaling and root planning can significantly improve periodontal health in periodontal disease patients, in comparison to patients subjected to scaling and root planning only.

In the present study, doxycycline 100 mg taken for 14 days produced more satisfactory outcomes in comparison to the group not subjected to the use of the drug. This particularly applies to gingival index and probing depth, which significantly decreased, and glycated hemoglobin values. When glycated hemoglobin values were assessed, it was found that they decreased to a greater extent in group 2. Additionally, final values were closer to reaching acceptable standards[3] of metabolic control in diabetic patients, when group 2 was compared to group 1. Such findings agree with a previous study[16] of which authors drew incisive conclusions based on experimental intervention on a group of Pima people comprising 113 individuals with type 2 diabetes and periodontitis. The group was subjected to mechanical therapy combined with a number of antimicrobial drugs. All groups undergoing treatment with doxycycline presented with decreased probing depth and subgingival *Porphyromonas gingivalis,* in comparison to a control group not subjected to the use of doxycycline. Additionally, the groups presented a significant decrease of 10% in glycated hemoglobin rates within a 90-day period. Such quite limited evidence suggests that untreated periodontal infection can increase the risk of diabetic patients having insufficient glycemic control and developing secondary systemic complications.[17]

Ciancio and Ashley[14] conducted experiments through which they have found that the adjunct use of doxycycline in low doses (20 mg) is effective and well tolerated, which might result in improvements not only regarding periodontal index, but also periodontal health. The use of doxycycline in low doses (20 mg) can inhibit tissue loss due to the anti-inflammatory action of this drug,[18] improve adult patients' periodontal health in case of individuals with periodontitis, in addition to reducing levels of gingival crevicular fluid, as well as preventing clinical attachment loss in adult patients with periodontitis.[13] It has been found that in cases of treatment carried out with doxycycline in low doses, microbial changes in one's microbiota associated with periodontitis in adult patients result from the anti-collagenase properties of the drug rather than its antimicrobial effects.[19] In the present study, microbiological analyses revealed a smaller amount of Gram-negative microorganisms in group 2 and a larger amount of Gram-positive ones in the same group, which is compatible with

gingival health standards. Taking the aforementioned outcomes into account, as well as those relative to plaque index, it can be inferred that doxycycline has a qualitative effect on the composition of bacterial plaque, since no statistically significant difference was found for plaque index.

Tetracyclines are bacteriostatic, bacterial protein synthesis inhibitors, have a broad spectrum, and are effective against Gram-positives and Gram-negatives, cocci, as well as aerobic and anaerobic bacteria. The following microorganisms are prevalent in periodontal disease: *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Tannerella forsythia*, and motile bacilli such as *Campylobacter rectus*. Gram-positives isolated are mainly streptococcus, actnomycetos and peptostreptococcus, all microorganisms comprising the spectrum of tetracyclines.[8]

Periodontal disease is found at the gingival sulcus/periodontal pocket; thus, the ability of antibiotics reaching the infection site should be considered. Importantly, antibiotic concentration in serum levels is as important as medication concentration in the gingival fluid. Tetracyclines and metronidazole present satisfactory penetration into the gingival sulcus fluid, with tetracyclines being able to reach higher concentrations in the fluid rather than in serum levels not only in natural teeth, but in implants as well.[8] Other authors[15] report that tetracycline concentration in the gingival fluid is greater than in blood.

In contrast, Sakelari et al[20] assessed the concentration of three different tetracyclines (tetracycline, minocycline and doxycycline) in the gingival crevicular fluid (GCF), plasma and saliva, and concluded that the highest concentration was found in blood plasma followed by GCF and saliva. In both tested fluids, doxycycline was found in higher concentrations, whereas tetracycline was found in lower concentrations. However, it is worth highlighting that *in vitro* studies might not exactly reproduce periodontal pocket conditions.[8]

The use of antibiotics might prove an adjunct to periodontal treatment, which does not eliminate the need for drainage, scaling and root planning or other periodontal procedures. Low-dose doxycycline administration proved effective in treating chronic periodontitis when combined with scaling and root planning. Nevertheless, there is a consensus that indiscriminate use of those drugs might lead to selection of resistant strains. Therefore, it is recommended that antibiotics be used with caution, so as to prevent the development of resistant microorganisms, which could worsen infection.[8]

CONCLUSION

Within the limits of the present study and based on the clinical significance of outcomes, it is possible to conclude that all assessed periodontal clinical parameters presented some degree of improvement, similarly to glycated hemoglobin levels in both treated groups, up to 180 days. Therefore, we suggest that systemic use of doxycycline combined with basic periodontal therapy revealed more beneficial and satisfactory clinical and microbiological outcomes in comparison to basic periodontal therapy alone, in individuals with type 2 diabetes and chronic periodontitis.

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