GENERALIZED AGGRESSIVE PERIODONTITIS: MICROBIOLOGICAL COMPOSITION AND CLINICAL PARAMETERS IN NON-SURGICAL THERAPY

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ABSTRACT

The aim of this study was to determine the variations in periodontal parameters and microbiological composition in periodontal pockets at the baseline and 3 and 6 months post-treatment in patients with Generalized Aggressive Periodontitis (GAP) undergoing non-surgical periodontal treatment combined with chlorhexidine and systemic antibiotics. Medical and dental history was taken from 10 subjects, average age 30.6±2.7 years, diagnosed with GAP. A non-surgical periodontal treatment combined with 0.12% chlorhexidine, 875 mg amoxicillin and 500 mg metronidazole every 12 hours for ten days was conducted. At each visit, the following measurements were recorded: bacterial plaque (BP), bleeding on probing (BOP), probing depth (PD), clinical attachment level (CAL), hypermobility, and furcation lesions, and a sample of subgingival plaque was taken from the site of the deepest probing depth of each sextant to identify Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Prevotella intermedia and Aggregatibacter actinomycetemcomitans using molecular biology techniques. After 6 months, the Wilcoxon test showed an increase of 0.97 mm in CAL (p=0.0047) and 2.54 mm in PD (p=0.009). A healthy site was defined as having a PD <5 mm, negative BOP and no pathogenic bacteria detected at 6 months, indicating significant improvement (p=0.008), with OR (95% CI) =4.7 (1.1022-20.11). With the treatment protocol used in this study, 6 months after treatment, patients had an approximately 4-fold higher possibility of presenting PD <5 mm and periodontal pockets without periodontal pathogenic bacteria.

Key words: *Aggressive periodontitis, periodontal treatment.*

PERIODONTITIS AGRESIVA GENERALIZADA: COMPOSICIÓN MICROBIOLÓGICA Y PARÁMETROS CLÍNICOS EN LA TERAPIA NO QUIRÚRGICA

RESUMEN

En este trabajo, nos propusimos determinar las variaciones de los parámetros periodontales y la composición microbiológica de las bolsas periodontales al inicio, a los 3 y 6 meses después del tratamiento en pacientes con periodontitis agresiva generalizada (GAP), sometidos a tratamiento periodontal no quirúrgico combinado con clorhexidina y antibióticos sistémicos. Se elaboró historia médica y dental en 10 sujetos, con una edad media de 30,6 ± 2,7 años, con diagnóstico de GAP. Se les practicó tratamiento periodontal no quirúrgico combinado con clorhexidina al 0,12%, 875 mg de amoxicilina y 500 mg de metronidazol. Los antibióticos se prescribieron cada 12 horas durante diez días. Se registraron: la placa bacteriana (BP), sangrado al sondaje (BOP), la profundidad de sondaje (PD), el nivel de inserción clínica (NIC), hipermovilidad y lesiones de furcación. En cada visita, se tomaron las mediciones, y se tomó una muestra de la placa subgingival en sitio de la mayor profundidad al sondaje en cada sextante para identificar mediante técnica de biología molecular: Porphyromonas gingivalis, Treponema denticola, forsythia Tannerella, Prevotella intermedia, y Aggregatibacter actinomycetemcomitans. Después de 6 meses, el análisis de la prueba de Wilcoxon mostró un aumento de 0,97 mm de CAL (p = 0,0047) y 2,54 mm en la PB (p = 0,009). Se definió sitio sano, cuando se determinó un PD <5 mm, BOP negativo, y no se detectaron bacterias patógenas a los 6 meses, lo que indicó una mejora significativa (p = 0,008), con (IC 95%) = 4,7 (1,1022 a 20,11). Con el protocolo de tratamiento presentado, es posible especular que a los 6 meses después del tratamiento, un paciente puede tener aproximadamente 4 veces más posibilidades de presentar una PD<5 mm y bolsillos periodontales sin bacterias patógenas.

Palabras clave: *periodontitis agresiva, tratamiento periodontal.*

INTRODUCTION

Generalized Aggressive Periodontitis (GAP) comprises a group of periodontal pathologies characterised by rapid evolution, severe bone loss, few clinical manifestations, low-grade inflammation and an amount of microorganisms which does not appear to be proportional to the severity of destruction [1]. The condition is diagnosed when

more than 30% of the teeth are affected, including at least three permanent teeth other than incisors and first molars, and is mainly diagnosed in systemically healthy persons at an early age [1]. It is also speculated that there is a hereditary component in susceptibility [2].

A highly virulent microbial flora has been described, with *Aggregatibacter actinomycetemcomitans* considered to be a risk factor because its persistence is associated with recurrence[3,4]. Other authors suggest that *Porphyromonas gingivalis, Tannerella denticola* and *Prevotella intermedia*, together with *A. actinomycetemcomitans*, are responsible for the low response to treatment in GAP patients because of a relationship between the rapid loss of periodontal insertion in patients undergoing maintenance and the persistence of these species [5,6].

The primary objective of treating this kind of periodontal disease is to modify the pathogenic subgingival microbiota to non-pathogenic and to prevent the reestablishment of virulent bacteria in the subgingival biofilm. These changes should improve clinical parameters such as bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL) [7,8]. The identification of the bacterial species that colonise the pockets might be a tool for predicting the outcome or success of treatment, especially with the use of additional antiseptic and antibiotic treatment [9, 10].

Several studies have reported the effectiveness and clinical safety of non-surgical periodontal therapy combined with systemic antibiotics for the treatment of GAP [11- 13]. However, there is no clear consensus on the choice of antibiotics or the protocol for the use and duration of antibiotic treatment. Moreover, several important issues related to the therapy have yet to be clarified. There is no previous report in Argentina about the effectiveness of non-surgical therapy combined with antibiotics such as amoxicillin and metronidazole for the management of periodontal flora in GAP. The aim of this study was to analyze the periodontal clinical parameters and the microbiological composition of periodontal pockets in patients diagnosed with GAP, and their response to non-surgical periodontal treatment combined with 12% chlorhexidine and 875 Amoxicillin plus Metronidazole 500 mg by comparing baseline to the situation at 3 and 6 months.

MATERIALS AND METHODS

Study Population

Ten unrelated patients of both sexes, aged 15 to 35 years, were recruited from the Department of Periodontics of the Independencia Foundation in the city of Cordoba, Argentina. Detailed systemic and oral anamnesis procedures were performed to assess the inclusion criteria, and GAP was diagnosed according to the definition of the American Academy of Periodontology [14]. The inclusion criteria for the patients were: 1) having at least 20 teeth, presenting interproximal bone loss in at least 3 teeth other than incisors and first molars and 2) being systemically healthy, having no previous history of periodontal treatment or history of prescribed antibiotic medication within the previous 6 months. Patients who were pregnant, breastfeeding, diabetic, smokers, immunosuppressed or drug addicts were excluded. Teeth with lesions with furcation were excluded. The Ethics and Discipline Committee of the Independence Foundation approved the protocol. All patients received a precise explanation of the research protocol, signed an informed consent to take part in the study, and were treated following the principles of the Helsinki Declaration.

Clinical Records and Treatment Protocol

A complete medical and clinical dental history, including serial radiographies and periodontal charting (Go- Probe* R), was performed on each patient. Researchers MMU and JM recorded all clinical parameters at the beginning of the protocol and at 3 and 6 months after treatment. The following clinical parameters were recorded: 1) presence/absence of supragingival bacterial plaque (BP), presence/absence of bleeding on probing (BOP), dental hypermobility, PD in mm, and loss of clinical attachment level (CAL) in mm. The measurements were taken from 6 different sites per tooth, excluding the third molar, using a Hu Friedy periodontal probe (PCP-UNC-15, Hu-Friedy, Chicago, IL). The inter-rater correlation coefficient for the PD measurements was 0.85, indicating a high level of reliability between MMU and JM.

Periodontal treatment consisted of detailed oral hygiene technique instructions, followed by root scaling and planing, and rinsing with a solution of 0.12% chlorhexidine digluconate (GlaxoSmithKline). Following their oral hygiene routine, patients were

instructed to rinse with 15 ml clorhexidine for 30 seconds twice a day for 60 days, beginning after the first session of mechanical debridement. Mechanical debridement consisted of progressive sessions of root scaling and planing once a week divided into approximately 5 to 7 sessions, depending on the case. The treatment was performed under local anaesthesia, by quadrant and with specific Gracey curettes (Hu-Friedy, Chicago, IL) for each sector and face of the teeth. The treatment was considered complete when BP was less 10%, there was no bleeding or any visible sign of inflammation, and no calculus was detected by the calculus detection device (Hu-Friedy, Chicago, IL). Patients received a prescription of 875 mg amoxicillin and 500 mg metronidazole every 12 hours for 10 days following the first scaling session. The dosage and duration of the antibiotic prescription are in relation to the pharmacological presentation. After completing the basic treatment, the clinical parameters were re-evaluated after 3 and 6 months with reinforcement of the hygiene instructions.

Sample Collection for the Microbiological Identification of Periodontal Pathogens

The samples for microbiological analysis were taken at the beginning of the non-surgical periodontal treatment and at 3 and 6 months. The material for the analysis was collected on the site presenting the deepest PD on each subgingival sector of each sextant, on the proximal faces: mesial-vestibular, mesiopalatal, distovestibular and distopalatal.

Prior to taking samples, the supragingival plaque was removed from the interproximal surfaces using a sterile curette. The surfaces were isolated with sterile gauze and five consecutive #35 paper points were inserted with catheterisation movements to the depth of the periodontal pocket for 60 seconds each. The cones were placed into Eppendorf tubes and stored at 4°C until processing.

Sample Processing

1- DNA extraction:

Two hundred microliters of sterile water were added to the Eppendorf tubes containing the paper points impregnated with the material extracted from the periodontal pockets. Each tube was incubated at 37ºC for 10 minutes and centrifuged at 14,000 g for 5 minutes. DNA was extracted using conventional techniques [15]. To verify the presence of DNA in the supernatant, electrophoresis was performed on 0.8% agarose gel stained with ethidium bromide and visualised using ultraviolet (UV) light.

2- Identification by conventional PCR:

First, we amplified a highly preserved specific sequence (960 bp) of the 16S rRNA gene to identify gram-negative bacteria in the sample. Once Gram-negative bacteria were confirmed in the sample, PCRs with specific oligonucleotides for *A. actinomycetemcomitans, T. forsythia, P. gingivalis, P. intermedia*, and *T. denticola* were performed following the protocol described by Ashimoto *et al* [16]. The assay was performed twice for each sample, including a negative control by adding to each reaction one tube without DNA and one tube with DNA from organisms isolated in a culture and donated by the Bacteriology Laboratory at the Reina Fabiola Hospital (Catholic University of Cordoba). The PCR products were analysed by electrophoresis on 1.6% agarose gels. The gels were stained with ethidium bromide and photographed under UV light. A molecular marker indicated the size of the amplification product in the gel (Perkin Elmer 100-bp Marker). For doubtful results, the samples were sequenced to corroborate the specificity of the amplified fragment (ABI PRISM 310, Applied Biosystems, USA).

Statistical analysis

Statistical analysis was performed using SPSS (Ver. 9; SPSS, Inc.) and Infostat vs2007e data analysis software. Significance was defined as p values under 0.05 ($p<0.05$). A sample size of 10 subjects was needed to provide an 80% power for detecting an average 0.75% reduction in probing depth between baseline and 6 months. Once patients had been selected for treatment, it was not possible to include additional patients to compensate for any potential dropouts. The intra-class correlation coefficients for mean PD and CAL were 0.92 and 0.91 respectively. The examiner's reproducibility of measurements taken at baseline and at 6 months was good (0.93 and 0.92 respectively).

The quantitative variables (PD, CAL) are expressed as means and standard deviations, and the qualitative variables BP, BOP, S and presence of bacteria in the pockets are expressed as absolute and percent frequencies. The statistical significance

between the mean values for the same periods of assessment was evaluated using the paired Student T test, and the differences between 2 periods of treatment were assessed using the Wilcoxon Signed Rank test. The presence of bacteria and the CAL and PD values at the end of the treatment were correlated with 2x2 contingency tables, using Pearson's chi-square test or Fisher's exact test when few samples were analysed. Subsequently, raw odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to calculate the relative risk of disease. Status was considered healthy when after six months, pathogenic bacteria were not detected, BOP was negative and PD<5 mm was recorded in the treated pockets.

RESULTS

This study analysed 60 periodontal sites in 4 males and 6 females, average age 30.6±2.7 years (range 21-35 years) who completed all stages of the project. Patients had an average 27 tooth, although one patient lost two teeth and another patient lost one tooth during the treatment. The complete oral examination revealed an average 42±7.9 pockets over 5 mm and 121.5±6.9 pockets under 5 mm. BOP was present in the upper maxilla in 47.6% of the cases, and in the mandible in 47.9%. Table 1 summarises the changes in periodontal parameters at the different time points of the assessment. Mean PD and CAL values in mm decreased significantly throughout the treatment in all patients (p<0.035). After six months post-treatment, according to the Wilcoxon Signed Rank test, there was a 0.97 mm increase in CAL (p=0.0047) and a 2.54-mm recovery in PD (p=0.009). Positive BOP decreased by 40.47% in the upper maxilla and by 37.2% in the lower maxilla after treatment. At six months, the number of periodontal pockets deeper than 5 mm decreased by 36.6%, whereas the frequency of pockets less than 5 mm deep increased significantly. Regarding the distribution throughout treatment of the five pathogens identified in the periodontal pockets at all sites, *P. gingivalis, T. denticola*, and *T. forsythia* were identified with greater frequency at the beginning of the treatment. *P. gingivalis* was not detected at the 3-month follow-up and only recognised at one site six months post-treatment, whereas *T. denticola and T. forsythia* persisted at all sites after completion of the 6-month period.

P. intermedia was identified in association with *A. actinomycetemcomitans* only at the beginning of the study. These bacteria were not isolated again at any site throughout the study. The absence of *P. gingivalis, P. intermedia* and *A. actinomycetemcomitans* after treatment concurred with the increase in non-virulent Gram-negative bacteria during the 3- and 6-month periodontal maintenance period (Table 2).

To analyse whether the reduction in PD observed at the 6-month follow-up was associated with

Table 1: Periodontal Clinical Parameters during the treatment.

	Baseline (n=60)	3m (n=60)	6m (n=60)	p
PD(mm)	7.0±2.6*	4.63±3.8	4.46±3.7	0.002
CAL(mm)	7.6±6.5*	6.8±4.5	6.63±5.4	0.035
Upper BOP (%)	47.6	42.9	4.047	0.007*
Lower BOP (%)	47.9	39.08	37.19	0.039*
Pockets>5mm	42*±0.9	25±0.2	22±0.3	0.037
Pockets<5mm	121*±6.4	137±0.1	140±04	0.045

The scores represent means ± standard error; in millimeters of probing depth (PD) and clinical attachment level (CAL), and the number of pockets of more than or less than 5 mm at all sites measured at each stage of treatment at 3m (3 months) and 6 m (6 months). BOP bleeding on probing; the numbers represents the percentage of the average score. *Significant p value between baseline and 6m.

Table 2: Distribution of Periodontal Bacteria at baseline and 3- and 6- months post-treatment.

	Pg Sites (%)	*Td* Sites (%)	*Tf* Sites (%)	*Pi* Sites (%)	*Aa* Sites (%)	*Others* Sites (%)
Baseline	13 (43.3)	15 (47)	13 (40)	12 (37)	4 (10)	2 (6)
3 months	0	6 (20)	7 (23)	0	0	18 (60)
6 months	1 (3)	5 (17)	8 (27)	0	0	16 (53)

The numbers represent the number of sites where each bacterium was identified; percentages between brackets. *Porphiromonas gingivalis (Pg), Treponema denticola (Td), Tannerella forsythia (Tf) Prevotella intermedia (Pi)* and *Agreggatibacter actinomycemcomitans (Aa)*, Others: Gram-negative bacteria.

the presence/absence of periodontal bacteria, a univariate analysis was performed to correlate the presence of 1 or more pathogenic bacteria with PD values compatible with clinical improvement. For this purpose, healthy status was considered to be PD<5 mm plus absence of pathogenic bacteria in the pocket and absence of BOP. The chi-square test revealed a statistically significant association of X^2=5.6, p=0.008, a statistically significant improvement between baseline and the 6-month measurement, with OR (95% CI) = 4.7 (1.1022-20.11). This finding indicated that the chance of pockets improving to PD<5 mm at 6 months post-treatment was 4-fold higher when periodontal pathogenic bacteria were absent from the periodontal pocket.

DISCUSSION

In our study, the analysis of clincal parameters before and after treatment revealed positive results for mechanical periodontal therapy combined with chlorhexidine and antibiotics in patients with GAP, showing significant improvements in clinical periodontal variables such as decreased PD, increased CAL, and decreased percentage of plaque and sites with BOP, which were maintained at the 6-month follow-up. Notably, the average increase in CAL was 0.97 mm at six months after the beginning of the treatment, and the PD decreased by 2.54 mm during the same period. This clinical success included a significant reduction in periodontal pathogens at the treated sites. These results are in agreement with other studies reporting that non-surgical treatment including the use of amoxicillin and metronidazole leads to clinical benefits and significant reduction in periodontal pathogens.[10- 13].

Hughes et al.[17] conducted a study on patients with GAP following treatment with superficial root debridement and oral hygiene instructions. In the re-assessment, they report a mean decrease of 2.11 mm in PD and an increase of 1.77 mm in CAL. They report that 32% of the patients with a negative response to treatment were smokers and suggest that smoking is the most important factor associated with treatment failure [17]. The fact that our study excluded smokers may explain our higher PD values after 6 months.

At the 3- and 6-month follow-ups we found significant reduction in the presence of *P. gingivalis, P. intermedia* and *A. actinomycetemcomitans* in the periodontal pockets treated, indicating the efficacy of mechanical therapy combined with 875 mg amoxicillin and 500 mg metronidazole every 12 hours for 10 days and Clorhexidine 0.12% rinses to eliminate pathogens and thereby restore periodontal tissue. It is also worth noting that the *T. denticola* and *T. forsythia* were identified at all stages of the treatment, and although they were detected in fewer pockets, the association between *T. denticola* and *T. forsythia* may be resistant to the combined treatment. Other authors have described the bacterial relationship between *P. gingivalis, A. actinomycetemcomitans* and *T. forsythia* in GAP [18]. We note that different studies use different doses and duration of the antibiotic treatment [11]. The amoxicillin dose used in our study has not been reported in other studies. For example, Gomes Baeta et al. [19] prescribed 500 mg amoxicillin and 250 mg metronidazole 3 times per day for 10 days, and detected *A. actinomycetemcomitans* in all samples up to the 9-month post-treatment follow-up session. Haffajee et al. [20] showed that systemic administration of these antibiotics may suppress periodontal bacteria efficiently and thereby improve the therapeutic response [18]. We believe that one weakness of the current study is that the high doses of amoxicillin used did not prevent the persistence of *T. denticola* or *T. forsythia* either at 3 or at 6 months. Further studies are needed to analyse whether a combination of amoxicillin/metronidazole might induce persistence or antibiotic resistance in the periodontal microflora. The risk of side effects or adverse events also remains to be addressed [12].

Mechanical instrumentation significantly changed the composition of the subgingival microflora by decreasing virulent microorganisms and increasing beneficial cocci and bacilli, with this bacterial pattern leading to a healthy bacteriological profile [20]. Some authors suggest a limited effect of root scaling and planning on certain pathogenic species because their complete elimination might be difficult as a result of re-infection of successfully treated sites. Johnson et al.[21] suggest that after 6 months, these bacteria recolonise the plaque when they remain in the epithelial cells of the oral mucosa and are unaltered by treatment.[21] These data support the hypothesis that extracrevicular bacterial deposits contribute to the development of recurrent diseases

in some patients [19,20]. We observed recolonization by *P. gingivalis* in 1 patient at the 6-month follow-up. According to our data**,** *P. gingivalis* in a periodontal pocket might be a risk factor for unsuccessful treatment or relapse. It is important to note that the reinforcement of oral hygiene and re-instrumentation at follow-up appointments contributed to the continuous reduction of certain pathogenic species. Our observations, as well as studies by other authors, have noted a significant decrease in the bacterial population in the treated pockets at three months, and repopulation at six months [22]. This repopulation may be caused by the use of systemic antibiotic therapeutic regimen. While adjunctive therapy is effective, few studies have reported whether periodontal species have developed resistance to antibiotics prescribed [18].

Because GAP is rarely diagnosed in our location, the sample size in our study is limited. Furthermore, the 6-month follow-up period might be too brief for longitudinal assessment. An increase in the number of patients and a longer follow-up period would enhance the study's strength and contribute to establishing appropriate intervals for maintaining periodontal health. Notably, although the clinical outcome was positive after 6 months, the elimination of certain bacteria was shown to be transitory. This persistence may contribute to the recurrence of the disease in some patients. To achieve long-term stability in clinical outcomes, it is essential to implement strict maintenance programmes. We believe that controlling the microbiological composition of the pocket may help reduce the use of antibacterial agents and the risk of producing bacterial resistance.

The rationale for adding amoxicillin to the prescription of this combined drug regimen is based on a synergistic effect of amoxicillin on metronidazole and its hydroxymetabolite against *A. actinomycetemcomitans* [23]. In our study, only 10% of the periodontal pockets were found to contain *A. actinomycetemcomitans* at baseline. Bazzano et al. report similar frequencies in patients with severe chronic periodontitis and treated only with root scaling and planning [24]. A study that evaluated metronidazole, alone or combined with amoxicillin, as an adjunct to nonsurgical therapy in chronic periodontitis subjects, showed that the clinical and microbiological benefits of both treatment options were very similar [25]. Further studies are needed to discuss the balance of the risk/benefits ratio of the prescription adjunctive antimicrobial regimen, in particular when high doses of amoxicillin are used (825mg/10 days/ 2 times a day/) and *A. actinomycetemcomitans* is identified in low frequency.

Our study verified that in patients diagnosed clinically, radiologically and microbiologically with GAP, virulent bacterial species may be eliminated by mechanical treatment combined with specific systemic antibiotics (875 mg amoxicillin and 500 mg metronidazole), antiseptics (such as 0.12% chlorhexidine digluconate) and a 6-month maintenance period. The above protocol leads to a significant recovery in periodontal parameters such as PD and BOP, achieving values that are compatible with periodontal health.

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