The Effectiveness of Adjunctive Systemic Antibiotics to Non Surgical Therapy in Chronic Periodontitis Patients

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Abstract: Background: The aim of this study was to assess the clinical and microbiological effects of moxifloxacin (MOX) and combined amoxicillin and metronidazole (AMX+ MET) as an adjunct to scaling and root planning (SRP) and to compare it to conventional mechanical periodontal treatment alone in patients with advanced chronic periodontitis by means of a species-specific sequences of DNA. **Methods:** Clinical parameters and subgingival bacterial plaque sample were collected from forty two subjects divided into three groups. Scaling and root planning (SRP) group, SRP with amoxicillin plus metronidazole and third group SRP with moxifloxacin. Data obtained before initial therapy, at 3 and 6 months after completion of therapy for evaluation of four different periodontopathogenic species using the quantitative PCR technique. **Results:** The results of the present study revealed that the three treatment modalities resulted in significant reduction in PI, GI, PD, and the CAL over time although there was no significant difference between groups at different follow up periods. There was statistically significant strong positive correlation between clinical parameters and bacterial count. **Conclusion:** This study confirms the efficiency of non surgical periodontal treatment to achieve reduction of the periodontal pockets and to ensure proper conditions for effective plaque control and stable levels of the periodontal attachment. Antibiotic prescribing should be the exception rather than the rule and only considered after conventional therapies have been unsuccessful.

[Riham Omar Ibrahim and Olfat Shaker. **The Effectiveness of Adjunctive Systemic Antibiotics to Non Surgical Therapy in Chronic Periodontitis Patients**. *J Am Sci* 2012;8(12):374-383]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 52

Keywords: Non surgical therapy, antibiotics, amoxicillin, metronidazole, moxifloxacin, PCR, bacteria.

1. Introduction

Chronic periodontitis (CP) is an inflammatory disease caused by groups of specific microorganisms which result in progressive destruction of the supporting structure of the teeth [(1)].

The periodontal microbiota is a complex community of microorganisms. Bacterial plaque is considered the principal etiological factor in the onset and progression of periodontitis [(2)]. Aggregatibacter actinomycetemcomitans (A.a), Porphyromonas gingivalis (P.g), Prevotella intermedia (P.i), and Tannerella forsythensis (T.f) are strong markers of periodontitis in adults [(3)] and these species have been linked to the progression of the disease [(4)]. In the microbiological diagnosis of periodontal diseases, subgingival plaque is used to detect and quantify bacterial species which are associated with moderate and advanced chronic periodontitis [(5)]. Strong positive associations and presence of P.g., P.i., T.f., and *Campylobacter rectus* were described in adult periodontitis [(6)]. While A.a., *Eikenella corrodens*, *Fusobacterium nucleatum*, and *Treponema denticola* were considered putative periodontopathogenic microorganisms [(7)].

A.a. and P.g. are implicated in the pathogenesis of periodontitis. These bacteria are able to produce virulence factors that act locally within the sulcus, and result in tissue destruction [(8,9)]. Virulence factors include proteolytic enzymes produced by P.g. and leukotoxins produced by A.a. [(9)]. *P. gingivalis* has shown the ability to invade human gingival fibroblasts in cell culture while both P.g. and A.a. have the ability to invade human oral epithelial cells in cell culture [(10)]. *P. intermedia* resist phagocytosis, probably by virtue of its capsule. P.i is an important periodontal pathogen, in association with P.g and A.a [(11)]. T. f. possesses several virulence factors including the production of a trypsin-like protease and lipopolysaccharide [(12)] as well as its ability to penetrate host cells and induce apoptosis [(10,13)].

P. gingivalis and *T. forsythia* are strong markers of periodontitis in adults, and have been linked to the progression of the disease [(3, 14)].

A microbiological diagnosis has been suggested to complement the clinical diagnosis and is used as an indicator for more extensive treatment modalities such as surgery or the administration of systemic antibiotics [(11,15)]. Microbiological laboratory

procedures have been involved in diagnosis and therapy control of severe forms of periodontitis for many years [16].

The Polymerase Chain Reaction (PCR) can specifically identify microorganisms in clinical samples by testing for the presence of species-specific sequences of DNA. It is specific, rapid and extremely sensitive, being able to detect even one copy of the searched DNA target and does not require rigorous conditions for transport of samples from the clinical department to the laboratory [17].

Ribeiro *et al.* 2008 [18] reported that PCR is a powerful diagnostic tool that can detect very low prevalence nucleic acid sequences with a high degree of accuracy in subgingival plaque samples. They proposed that PCR should be regarded as the "gold standard" for determining the actual impact of the treatment protocols on bacterial load especially when the threshold number of bacteria is considered. In clinical trials, identification of A.a., P.g., T.f. and Treponema denticola is used to analyze periodontal therapy [19].

The prime goal of successful periodontal therapy is to eradicate the periodontopathic microorganisms, or inhibit their colonization. The objectives of nonsurgical therapy are the reduction of the bacterial load, the alteration of the microbial composition towards a flora associated with health. Microbiologic monitoring during treatment can be an aid in understanding whether this is efficient and whether patients respond to therapy. As a microbiologic detection system, PCR-based approaches provide a sensitive and reliable method for identification and monitoring treatment of periodontal pathogens. Scaling and root planning (SRP) is the most commonly used periodontal therapy [20]. However, it is also evident that various subject-related and tooth - related factors may compromise the healing response to treatment [21,22].

SRP alone may not affect invading microorganisms that had penetrated the gingival tissue, so antibiotics provide a useful adjunct. The adjunctive use of systemically administered antibiotics has been shown to provide a better clinical outcome, particularly in terms of pocket depth reduction and attachment-level gain, than scaling and root planning alone in cases of chronic periodontitis [19,23]. Commonly used antibiotics include amoxicillin (AMX) and metronidazole (MET) [23,24]. Also, positive responses have been reported with systemic moxifloxacin (MOX) [19]. Moxifloxacin (MOX) has shown *in vitro* activity against gram negative enteric rods (GNER) [25], and *in vitro* [26] and *in vivo* [19] efficacy against periodontopathogens.

The purpose of this study was to assess the clinical and microbiological effects of moxifloxacin (MOX) as an adjunct to SRP and to compare it to SRP and SRP combined with amoxicillin and metronidazole (AMX+ MET) in patients with advanced chronic periodontitis by means of a species-specific sequences of DNA.

2. Patients and Methods:

Study Population:

Forty two subjects (age range: 38-62 years) were selected from those attending the outpatient clinic, Department of Oral Medicine, Periodontology and Oral Diagnosis, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt, between January 2011 and February 2012. This was a randomized clinical trial with 6 months of follow-up. Subjects were randomly assigned by a coin toss to receive one of the three treatments. The assignment of subjects to the treatment groups was carried out by the clinic coordinator remote from the study. The randomization code was held centrally by the clinic coordinator and was not broken until completion of the data analysis. In this study patients diagnosed with chronic periodontitis were divided into 3 groups: Group 1 (negative control group) which included 14 patients treated with scaling and root planning (SRP) alone. Group 2 (positive control group) included 14 patients treated with SRP and amoxicillin (Amoxil®: Medical Union Pharmaceuticals, Egypt) (500 mg every 8 hours for 10 days) plus metronidazole (Flagyl®: Pharco Pharmaceuticals, Egypt) (250 mg every 8 hours for 10 days). Group 3 (test group) included 14 patients treated with SRP and moxifloxacin (Avalox®: Bayer Health Care, Germany) (400mg once daily for 10 days).

Detailed medical history of each subject was obtained according to the detailed questionnaire of the modified Cornell Medical Index [27].

Inclusion Criteria:

All patients were diagnosed with moderate-to-advanced generalized chronic periodontitis based on the clinical and radiographic criteria proposed by the 1999 World Workshop for Classification of Periodontal Diseases and Conditions [28]. The criteria for entry were a minimum of 14 natural teeth, excluding third molars, with at least five to six teeth had sites with probing depth ≤6 mm and attachment loss ≤5 mm and radiographically determined bone loss.

Exclusion Criteria

Exclusion criteria were: allergies to penicillin or quinolones pregnancy, lactation, current smoking or even within the last 5 years. All patients did not have

any systemic illness that could affect the progression of periodontal disease and had not received any periodontal therapy within the past 12 months and/or antibiotic, non-steroidal anti-inflammatory therapies during the previous 6 months prior to the study. Subjects with periapical pathology, orthodontic appliances, multiple systemic complications of DM, and under hormone-replacement, calcitonin, and alendronate therapies were also excluded from the study. The study protocol was approved by the Ethic Committee of Faculty of Oral and Dental Medicine. And it was explained to all participants, and informed consent forms were signed.

Clinical Monitoring

Clinical examination was performed by one calibrated examiner. For each patient, individual number of teeth present, excluding the third molar and the diagnostic periodontal clinical parameters was documented. The following periodontal parameters were evaluated on study sites: plaque index (PI) [(29)], gingival index (GI) [(30)], probing depth (PD), clinical attachment loss (CAL) using William's graduated periodontal probe to the nearest 0.5 mm. Full mouth periapical radiographs were taken for each patient to confirm diagnosis of chronic periodontitis.

Bacteriological sample collection:

Careful removal of supragingival biofilm, areas was washed with a water spray, isolated with cotton rolls and gently dried [(31)]. Then a sterile endodontic paper point (no. 35) was inserted into the bottom of the periodontal pocket for 30 seconds. The paper points then placed into sterile Eppendorff tubes containing 0.5ml of phosphate-buffered saline (PBS) and immediately stored at -20^{o}C till use. For each patient one transportation vial was loaded containing four paper points pooled from the 4 deepest sites of 4 teeth, one in each quadrant. This pooled plaque sample collected was for the evaluation of the content of the four subgingival species including Aggregatibacter actinomycetem comitans (A.a), Porphyromonus gingivalis (P.g.), Prevotella intermedia (P.i.) and Tannerella forthysia (T.f.) at baseline, 3 and 6 months after completion of therapy.

Therapy phase:

Full mouth SRP was performed by the same experienced periodontist using universal curettes and ultrasonic scalers in four visits over 2 weeks, quadrant in each visit after local anesthesia was given. The endpoint of SRP was tactile smooth root surface felt with an explorer tip.

All patients were instructed to maintain thorough oral hygiene measures consisting of Bass' brushing technique, the correct use of dental floss and an interdental brush. Subjects were recalled every two weeks during the first month and at 2 months interval up to the 6 months evaluation. In every maintenance session professional supragingival plaque control and reinforcement of oral hygiene motivation and instructions were performed. The microbiologist who analyzed subgingival samples was not aware of the treatment that the patient had received.

Clinical and microbiological monitoring was performed at baseline and at 3 and 6 months after SRP. Antibiotics were started on the first day of the initial phase.

Molecular Biology Methods:

Subgingival plaque samples were centrifuged at 3,000 Xg for 10 min. Supernatant was removed except 30 µl was left in each Eppendorff tube for DNA extraction. We studied various regions of 16S ribosomal RNA sequences in the GenBank database to find primer pairs which would be specific for all known strains of A.a, P. gingivalis, P. Intermediate, and T.f. The selected primers (Gibco BRL, São Paulo, SP, Brazil) were able to hybridize only with their specific target sequences.

PCR for Microbiological Detection

DNA was extracted from crevicular fluid using the DNA extraction Kit (Roche, Mannheim, Germany) according to the manufacturer's recommendations. The polymerase chain reaction (PCR) amplification of the conserved region of 16S ribosomal DNA was tested for periodontal pathogens including A.a, P.g., P.i. and T.f.

Table 1: Sequences and expected product size for PCR primers [(32,33,34)]

Primers	Sequences	PCR product size (bp)
A.a	5'-GCTAATACCGCGTAGAGTCGG-3' 5'-ATTTCACACCTCACTTAAAGGT-3'	443
P.g	5'-AGGCAGCTTGCCATACTGCG-3' 5'-ACTGTTAGCAACTACCGATGT-3'	443
P.i	5'-TTTGTTGGGGAGTAAAGCGGG-3' 5' TCAACATCTCTGTATCCTGCGT-3'	575
T. f	5'-GCGTATGTAACCTGCCCGCA-3' 5'-TGCTTCAGTGTCAGTTATACCT-3'	641

PCR Mixture

Amplification reactions were performed in a total volume of 50 µl consisting of: 100 ng of genomic DNA, 1µmol/L of the specific primers of the four types of bacteria (each in separate tube), 2.5U of Taq polymerase and 0.2mmol/L of dNTPs.

PCR amplification cycling condition:

PCR was performed for 35 cycles of 30 seconds at 95°C, 30 seconds at (56°C for A.a, 55°C for P.g, 57°C for P.i, and 55°C for T.f) and 60 seconds at 72°C in thermocycler. Twenty µL of each PCR reaction mixture was electroforesed in 2% agarose gel in tris acetate EDTA (TAE) buffer, and the amplification products were visualized under ultraviolet light, on ethidium bromide-stained gel.

Agarose gel electrophoresis:

The PCR products were visualized after electrophoresis on 2% agarose gel (containing 1 ug/mL ethidium bromide) by illumination with UV light.

Quantitation of PCR product:

PCR products were then quantitated by using a quantitation kit. This method depends on purification of the PCR using Promega Wizard PCR preps DNA purification kit (Promega Corporation, Madison, WI, USA). The mixture for quantitation consisted of DNA quantitation buffer, sodium pyrophosphate, NDPK enzyme solution, T4 DNA polymerase and DNA. All these contents were incubated at 37°C for 10 min. Then, 100 µL of Enliten L/L reagent was added. Immediately, the reaction was read using a luminometer. The same steps were done on DNAs of known concentrations provided by the kit, and a standard curve was performed by plotting the readings of the luminometer against the concentrations. Then, the readings of the amplified PCR product after using the luminometer were read from the standard curve. The results were expressed as pg/gm tissue [35].

Statistical analysis

Statistical analyses were performed using a commercially available software computer program (SPSS 20). Data were first examined for normality by the Kolmogorov-Smirnov test, and data that did not achieve normality were analyzed using non-parametric methods.

One way ANOVA test was used to compare age between groups and Chi square test was used to compare gender between groups. One way ANOVA and Kruskal-Wallis Test for non parametric data was used to compare clinical parameters between groups at different follow up periods.

ANOVA for repeated measures, Friedman and Wilcoxon Signed Ranks Test as post hock test were used to compare between follow up periods within groups. Kruskal-Wallis Test was used to compare percentage of bacterial reduction between groups at different follow up periods. Spearman correlation was used to determine significant correlation between clinical parameters (data) and bacterial counts.

3. Results

Table 2: Demographic characteristics

There was no sig. difference between groups regarding age (one way ANOVA, f (2, 41) =2.2, p>0.05) and gender (x^2 (2)=2.3, p> 0.05)

	SRP (n=14)	SRP +Amoxicillin & metronidazole (n=14)	SRP+Moxifloxacin (n= 14)
Age			
Mean ± Std.	50.57 ±10.6	50.7 ±6.8	44.57 ±8.8
Range	29	22	21
Gender (n)			
Male	10	6	8
Female	4	8	6

Table 3: Clinical parameters of the periodontal sites being selected for subgingival plaque sampling.

	SRP without antibiotic mean±sd (median)	Amoxicillin & metronidazole mean±sd (median)	Moxifloxacin mean±sd (median)
Plaque index			
Base line*	1.28 ±0.35 (1.25)$^{a+}$	1.7 ±0.4 (1.5)$^{a+}$	1.39 ±0.34 (1.5)$^{a+}$
3 month$^{\$}$	0.93 ±0.19 (1)$^{b+}$	1.2 ±0.37 (1)$^{b+}$	1.14 ±0.4 (1)$^{b+}$
6 month$^{\$}$	0.6 ±0.4 (0.75) $^{b+}$	1.0 ±0.5 (1)$^{b+}$	1.1 ±0.4 (1)$^{ab+}$
Gingival index			
Base line	1.67 ±0.27 (1.75)$^{+}$	1.67 ±0.41 (1.5)$^{a+}$	1.7 ±0.38 (2)$^{a+}$
3 month$^{\$}$	1.1 ± 0.2 (1)$^{+}$	1.18 ±0.37 (1)$^{b+}$	1.3 ±0.35 (1.25)$^{b+}$
6 month$^{\$}$	0.7 ±0.49 (1)$^{+}$	1 ±0.58 (1)$^{b+}$	1.14 ±0.2(1)$^{b+}$
Probing Depth			
Base line	3.44 ±0.3 (3.5)$^{+}$	3.77 ±0.36 (3.75)$^{+}$	3.77 ±0.56 (3.5)^{a}
3 month	2.96 ±0.26 (3)$^{+}$	3.1 ±0.37 (3.125)$^{+}$	2.95 ±0.54 (3)^{b}
6 month$^{\$}$	1.8 ±1.24 (2.5)$^{+}$	2.39 ±1.1(2.6)$^{+}$	2.73 ±0.55 (2.6)^{b}
Clinical Attachment Loss			
Base line	4.7 ±0.67 (4.7)$^{+}$	4.8 ±0.77 (4.6)^{a}	5.6 ±0.67 (4.8)^{a}
3 month$^{\$}$	4.5 ±0.69 (4.25)$^{+}$	4.4 ±0.99 (4.125)^{b}	5.2 ±1.8 (4.6)^{b}
6 month	2.98 ±2.01 (3.75) $^{+}$	3.7 ±1.99 (3.8)^{ab}	5.1 ±1.9 (4.7)^{b}

Table 3 summarizes clinical outcomes over time in the 3 groups. PI, GI, PD, and the CAL of sites underwent a significant reduction over time in all groups with no significant difference between groups at different follow up periods.

Plaque index

There was decrease in plaque index in both SRP group and AMX/ MET through follow up periods and difference was statistically significant between baseline and 3 month and between base line 6month (x^2 (2)=16.8, p<0.001) and (x^2 (2)=24.1, p<0.001) respectively. There was decrease in plaque index in MOX group through follow up periods which was statistically significant between insertion and 3 month. (x^2 (2)=11.6, p<0.01). There was significant difference between groups at base line f(2,41)=5.2,p<0.01) Tukey post hock test revealed sig difference between SRP group and Moxifloxacin group.

Gingival index

There was decrease in gingival index in SRP group through follow up periods and difference was statistically sig between all follow up periods. (x^2 (2)=26.08, p<0.001). There was decrease in gingival index in both AMX/ MET group and MOX group through follow up periods and diff was statistically sig between base line and 3month and between base line and 6 month (x^2 (2)=26.2, p<0.001) and (x^2 (2)=18.4, p<0.01) respectively. There was no statistical sig difference between groups at different follow up periods.

Probing depth

There was decrease in probing depth in both SRP group and AMX/ MET group through follow up periods and difference was statistically sig between all follow up periods (x^2 (2)=28, p<0.001) and (x^2 (2)=27.1, p<0.001) respectively.

There was decrease in probing depth in MOX group through follow up periods and difference was statistically significant between base line and 3month and between base line and 6 month (f(2)=29.96, p<0.001). There was no statistical sig difference between groups at different follow up periods.

Clinical Attachment Loss

There was decrease in clinical attachment loss in SRP group through follow up periods and difference was statistically significant between all follow up periods. (x^2(2)=20.5, p<0.001). There was decrease in clinical Attachment Loss in AMX/MET group through follow up periods and difference was statistically significant between base line and 3 month. (f (2)=6.1, p<0.01). There was decrease in CAL in the 3 groups through follow up periods and diff was statistically sig between base line and 3month and between base line and 6 month. (f (2)=29.9 p<0.001). There was no statistical sig difference between groups at different follow up periods.

Table 4: Bacterial count at Base line, 3 month, and 6 month

	SRP without antibiotic Median (IQR)	Amoxicillin & metronidazole Median (IQR)	Moxifloxacin Median (IQR)
Aggregatibacter actinomycetemcomitans (n)	6	6	8
Base line			
3 month	1155(848)	858(383)	1154(920)
6 month	0a	307(500)a	564(365)b
	0	0(150)	0
Porphyromonas gingivalis (n)	8	12	6
Base line	803(285)	845(358)	620(509)
3 month	403(455.5)a	370(455)a	0b
6 month	0	0	0
Prevotella intermedia(n)	4	4	10
Base line	1071(172)	1120(336)	2055(856)
3 month	0	268(537)	578(482)
6 month	0	0	0
Tannerella forsythia(n)	6	6	8
Base line	1055(172)a	910(856)a	1745(1295.75)b
3 month	0a	0(537)a	648(336)b
6 month	0	0	0(112.5)

Kruskal-Wallis Test followed by Mann-Whitney Test with bonferroni correction The interquartile range (IQR)

A.a. at 3 months SRP and AMX/MET group showed better results at 3 months while at 6 months there was no statistical difference between groups. P.g. at 3 months moxifloxacin showed better results

at 3 months while at 6 months there was no statistical difference between groups. No significant difference in P.i. between groups at 3 and 6 months. T.f. at 3 months SRP and AMX/MET group showed better results at 3 months while at 6 months there was no statistical difference between groups however MOX group showed increase in bacterial count at baseline.

Table 5: Percentage of bacterial count reduction

	SRP without antibiotic Median (IQR)	Amoxicillin & metronidazole Median (IQR)	Moxifloxacin Median (IQR)
Aggregatibacter actinomycetemcomitans (n)	6	6	8
Base line to 3 month	100%(0)a	75.2%(44)ab	43%(16)b
3 month to 6 month		85%(30)a	100%(0)a
Porphyromonas gingivalis (n)	8	12	6
Base line to 3 month	49.9%(18.5)a	59.8%(51)a	100%(0)b
3 month to 6 month	100%(0)a	100%(14)a	
Prevotella intermedia(n)	4	4	10
Base line to 3 month	100%(0)a	79%(41)a	62%(10)a
3 month to 6 month		100%(0)a	100%(0)a
Tannerella forsythia(n)	6	6	8
Base line to 3 month	100%(0)a	100%(31)ab	56%(13)b
3 month to 6 month		100%(0)a	95%(5)a

Kruskal-Wallis Test followed by Mann-Whitney Test with bonferroni correction The interquartile range (IQR)

There was no significant difference between AMX/MET and MOX groups in percentage of bacterial reduction from 3 months to 6 months.

A.a., P.g. and T.f showed statistical significant difference between the 3 groups in percentage in bacterial reduction from base line to 3 months. There was significant difference between SRP and MOX group in A.a. and T.f (100%) and between MOX group and other groups in P.g. (100%). P.i. showed no significant difference between groups in percentage in bacterial reduction from base line to 3 months.

Table 6: Correlation between clinical parameters and bacterial count

	Aggregatibacter actinomycetemcomitans	*Porphyromonas gingivalis*	*Prevotella intermedia*	*Tannerella forsythia*
Plaque index				
Correlation coffecient	0.673	0.216	0.568	0.27
P value	0.000*	0.188	0.002*	0.149
Gingival index				
Correlation coffecient	0.662	0.593	0.615	0.408
P value	0.000*	0.000*	0.001*	0.025*
Probing depth				
Correlation coffecient	0.524	0.76	0.529	0.538
P value	0.002*	0.000*	0.005*	0.002*
Clinical attachment loss				
Correlation coefficient	0.2	0.205	0.231	0.06
P value	0.281	0.21	0.246	0.75

Spearman correlation

There was statistically significant strong positive correlation between both A.a. and P.i. and plaque index, gingival index and probing depth. Also, there was statistically significant strong positive correlation between both P.g and T.f. and gingival index and probing depth.

4. Discussion

This randomized, clinical trial evaluated the clinical and microbiological effects of SRP with adjunctive antibiotics (MOX versus AMX/MET) in the treatment of subjects with chronic periodontitis.

Most of the variations in periodontal therapy outcomes result from factors acting at the site level. This agrees with previous studies that also assessed the relative contribution of multilevel variation for the outcome of periodontal therapy in clinical trials [21,22] which found out those site-level factors had a much greater impact than subject-level factors. The relative value of site-level factors becomes very important since indicators at this level have demonstrated strong associations with future periodontal attachment loss [36].

Scaling and root planning refers to the debridement of the roots removing plaque, endotoxin, calculus and other plaque-retentive local factors. The major role of non-surgical therapy is to reduce the quantity (mass) of bacterial plaque to a level (critical) that results in a balance between the residual microbes and the host response. As probing depth increases, instrumentation becomes less effective in removing the cause of the problem [37].

The reduction of the probing depth is one of the fundamental goals of the periodontal therapy. This study confirms the efficiency of the initial periodontal treatment to achieve reduction of the periodontal pockets and to ensure proper conditions for effective plaque control and stable levels of the periodontal attachment.

The prevalence of the residual pockets with probing depth greater than 4 mm determines the risk of disease progression. The reduction of the periodontal sites with PD above 7mm could limit the necessity of periodontal surgery [38].

The treatment modalities resulted in a reduction of the total bacterial count in the three groups, which was not statistically significant. This is in agreement with other reports which showed that manual instrumentation is able to lower the number of selected periodontal pathogens, as P.g., T.f. and T. denticola but is unlikely to eliminate these species from any subject [37,39,40]. Manual instrumentation decreases the population of gram-negative bacteria and allows for an increase in the population of gram-positive microbes. This shift is usually associated with an improvement in clinical parameters, such as decreased PD or bleeding on probing (BOP) [41].

The incomplete elimination of periodontal pathogens by non-surgical therapy can be explained by the ability of these bacteria to invade periodontal tissues, and their capacity in evading the host defense, thus causing tissue breakdown.

Not all patients or all sites respond uniformly and favorably to conventional mechanical therapy. Given the infectious nature of periodontal disease and the limited results that can be achieved with conventional mechanical therapies, the use of antibiotics is warranted for certain forms of periodontitis. Other studies that indicate that systemically administered antibiotics provide greater benefit in subjects with more periodontal disease and at deeper periodontal sites [41,42]. Systemically administered antibiotics can reach pathogens that are inaccessible to scaling instruments.

The β-lactams, including amoxicillin are broad spectrum drugs that show excellent tissue distribution but relatively low concentrations are found in GCF [43]. Winkel *et al.,* 1998 [44] reported increased frequency of amoxicillin and metronidazole resistance. Metronidazole has been reported as an effective agent for treating refractory periodontitis involving P.g. and/or P.i. [45].

Guerrero *et al.,* 2005 [46] clearly demonstrated that the systemic administration of a combination of metronidazole and amoxicillin, in conjunction with nonsurgical treatment of aggressive periodontitis, significantly improved clinical results for a period of six months.

The fact that metronidazole has a number of unpleasant side effects that are not well tolerated by some patients. Amoxicillin is definitely contraindicated in patients with penicillin hypersensitivities. Furthermore, the overuse, misuse and widespread prophylactic application of antimicrobial drugs are some of the factors that have led to the emergence of drug resistant microorganisms [47]. Also, conflicting results about clinical benefits of AMX/MET combination used as an adjunct to SRP means that alternatives should be investigated.

MOX inhibit bacterial DNA topoisomerase II and produce bactericidal effects against a broad spectrum of bacteria [48]. Studies [19,25,26] have outlined a procedure for clinical trials to investigate the effects of MOX in the treatment of patients with chronic periodontitis harboring GNER in subgingival plaque.

The present investigation demonstrated that the three treatment modalities resulted in significant reduction in PI, GI, PD, and the CAL over time although there was no significant difference between groups at different follow up periods. There was statistically significant strong positive correlation between clinical parameters and bacterial count (Table 6). It is important to point out the pivotal role of the supportive periodontal care. Plaque levels were maintained at a low level through the study in the 3 treatment groups. These results come in accordance with Gunetsch *et al.,* (2008) [19] study which was

conducted on 92 subjects with sever chronic periodontitis treated with SRP alone or in conjunction with either MOX or doxycyclin.

In the present study, there was no significant difference between amoxicillin/metronidazole and moxifloxacin group in percentage of bacterial reduction after 3 months and after 6 months. This is in accordance with the study by Ardila *et al.,* (2010) [25].

Darby *et al.,* (2005) [49], demonstrated significant decrease in percentage of P.i., T.f., and Treponema denticola in chronic periodontitis following SRP.

Milazzo *et al.,* (2002) [50] reported the *in vitro* activity of MOX compared to AMX, MET, erythromycin, clindamycin and cefoxitin against periodontal infections. Highest inhibitory concentration values of MOX were effective against P.g., P.i., actinomyces and fusobacterium nucleatum. They concluded that MOX produced bactericidal effects at 8 hours against periodontal pathogens.

Haffajee *et al.,* 1997 [51] reported significant decrease in prevalence and levels of P.g., T. f., and *Treponema denticola* with significant increase in levels of beneficial species in subjects with chronic periodontitis treated with SRP monitored at 3 and 6 months post therapy.

Small number of P.g., T.f., and *Treponema denticola* may exist in subgingival biofilms and increased in sites with higher probing depth and bleeding on probing [52]. *P. gingivalis* was highly susceptible to moxifloxacin and amoxicillin/clavulanic acid. These findings are in agreement with previous studies which have found that *P. gingivalis* is highly susceptible to these antibiotics [19,24,53]. Previous studies of the susceptibility of *A. actinomycetemcomitans* have also shown high susceptibility to these two antibiotics [19,23,25, 53]. Metronidazole in combination with amoxicillin has been shown to be successful in the treatment of A.a. associated periodontal disease [23].

Owing to geographical differences as well as differences over time, we suggest that the overuse and misuse of antibiotics could be influencing the manifestation of more highly resistant strains associated with periodontal infections in our population. Ongoing longitudinal surveillance studies have been crucial to the detection and monitoring of regional antimicrobial resistance patterns, and continue to provide important insights that may serve to modify local prescribing guidelines.

Conclusions

This study confirms the efficiency of the initial periodontal treatment to achieve reduction of the periodontal pockets and to ensure proper conditions for effective plaque control and stable levels of the periodontal attachment.

There is no single periodontal therapeutic regimen that will provide a beneficial response for all patients. It is very unlikely that there ever will be. Prescription of systemic antibiotic therapy in periodontics should be based upon scientific data. Antibiotic prescribing should be the exception rather than the rule and only considered after conventional therapies have been unsuccessful. Thus, antibiotics should always be prescribed after microbial culture identification and antibiotic sensitivity determination in periodontitis patients.

The authors report no conflicts of interest related to the study.

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11/5/2012