RESEARCH

Effect of adjunctive amoxicillin/metronidazole treatment on the recolonization levels of subgingival *Tannerella forsythia*, *Prevotella intermedia* and *Fusobacterium nucleatum* in periodontitis patients

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ABSTRACT

Effect of adjunctive amoxicillin/metronidazole treatment on the recolonization levels of subgingival *Tannerella forsythia*, *Prevotella intermedia* and *Fusobacterium nucleatum* in periodontitis patients

Background: The use of antibiotics adjunctive to non-surgical periodontal treatment is an accepted approach in the treatment of periodontitis. However, bacterial recolonization is a major drawback which complicates the maintenance of therapeutic acquisitions. The aim of this study was to evaluate the clinical and microbiological effects of adjunctive amoxicillin and metronidazole combination in the treatment of periodontitis during 6 months follow-up.

Materials and Methods: Twenty-two periodontitis patients were assigned as test (n=12) and control (n=10) groups. While test group received amoxicillin and metronidazole combination as an adjunct to scaling and root planing, control group treated with scaling and root planing alone. Clinical examinations and subgingival dental plaque sampling were conducted at baseline and 1, 3 and 6. months during the follow-up. *Fusobacterium nucleatum*, *Tannerella forsythia* and *Prevotella intermedia* levels were determined using real time polymerase chain reaction method.

Results: Both groups exhibited significant improvements in each follow-up period when compared to baseline. However, clinical improvements in test group were more prominent than control group as 3rd and 6th months. While some decrease was notable in the counts of all investigated bacteria in both groups, only *T. forsythia* levels significantly reduced at all follow-up months in test group comparing with controls.

Conclusion: The results of this study indicated that amoxicillin/metronidazole treatment adjunctive to scaling and root planing provided additional clinical benefits and suppressed the *T. forsythia* recolonization.

KEYWORDS

Amoxicillin, metronidazole, periodontitis, real-time PCR

ÖZ

Periodontitis hastalarında ilave amoksisilin/metronidazol tedavisinin subgingival Tannerella forsythia, *Prevotella intermedia* ve *Fusobacterium nucleatum* rekolonizasyon seviyelerine etkisi

Amaç: Periodontitis tedavisinde cerrahi olmayan periodontal tedavi ile eş zamanlı antibiyotik uygulamaları kabul görmüş bir yaklaşımdır. Bununla beraber, bakteri rekolonizasyonu tedavi kazanımlarının idamesini güçleştiren bir durumdur. Bu çalışmanın amacı, periodontitis tedavisinde amoksisilin ve metronidazol kombinasyonunun tedavi sonrası 6 aylık dönemde klinik ve mikrobiyolojik etkilerinin değerlendirilmesidir.

Gereç ve Yöntemler: Yirmi iki periodontitis hastası test (n=12) ve kontrol (n=10) gruplarına ayrıldı. Test grubu diştaşı temizliği ve kök yüzeyi düzleştirmesi işlemlerine ilaveten eş zamanlı amoksisilin ve metronidazol tedavisi alır iken, kontol grubuna yalnızca diştaşı temizliği ve kök yüzeyi düzleştirmesi uygulandı. Klinik değerlendirmeler başlangıçta ve 1, 3 ve 6. takip aylarında gerçekleştirildi. *Fusobacterium nucleatum*, *Tannerella forsythia* ve *Prevotella intermedia* seviyeleri gerçek zamanlı polimeraz zincir reaksiyonu yöntemi ile belirlendi.

Bulgular: Her iki grupta da bütün takip aylarında başlangıca göre anlamlı düzelmeler gözlendi. Bununla beraber, test grubunda 3. ve 6. aylarda kontrol grubunda göre daha fazla düzeyde klinik iyileşmeler tespit edildi. Her iki grupta da incelenen bakterilerin miktarlarında belirli düşüşler görülse de yalnızca *T. forsythia* seviyeleri tüm takip aylarında test grubunda kontrol grubuna göre anlamlı seviyelerde düşüş gösterdi.

Sonuç: Bu çalışmanın sonuçları, diştaşı temizliği ve kök yüzeyi düzleştirmesine ilaveten uygulanan amoksisilin/metronidazole tedavisinin ilave klinik faydalar ortaya koyduğunu göstermektedir. Ayrıca, destekleyici antibiyotik tedavisi *T. forsythia* rekolonizasyonunu baskılamıştır.

ANAHTAR KELİMELER

Amoksisilin, metronidazol, periodontitis, real-time PCR

Periodontitis is an inflammatory disease leading the loss of supporting tissues of the teeth. Although a wide variety of immunologic and genetic risk factors reportedly determine the severity of periodontal breakdown, bacterial dental plaque is the major etiological factor for the occurrence of periodontitis.[1] In addition, the presence of highly virulent pathogens in subgingival dental plaque aggravates the inflammatory host response and triggers periodontal breakdown.[2] Besides, synergetic bacterial interactions

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affect microbial diversity in dental plaque. Accordingly, bacterial variety may influence the severity periodontal destruction.[3] *Fusobacterium nucleatum*, a Gram-negative bacterium, plays an important role in microbial diversity of bacterial dental plaque by enabling multiple coaggregations. With this aspect, *F. nucleatum* is called as "bridge bacterium" due to close interactions with numerous pathogens.[4] *Tannerella forsythia* a Gram-negative anaerobe is another periodontal pathogen which is reportedly associated with periodontal breakdown.[5] Synergistic retaliations between *T. forsythia* and *F. nucleatum* have been documented.[6] As being another Gram-negative periodontal pathogen, *Prevotella intermedia* reportedly exhibit close interactions with *F. nucleatum*.[7]

Maintenance of obtained therapeutic periodontal outcomes is a challenging task for clinicians in the treatment of periodontitis.[8] Mechanical removal of disease-causing periodontal pathogens and associated factors by non-surgical periodontal therapy is the primary approach in the treatment process. Scaling and root planning (SRP) is an important treatment modality for the disruption of the biofilm and accompanying factors to obtain clinical benefits. However, the presence of deep periodontal pockets and anatomical conditions complicating the mechanical instrumentation such as furcation involvements reduces the efficacy of SRP.[9]

Bacterial recolonization directly affects the long-term success of periodontal treatment, as reported. Recolonization of residual microorganisms in the periodontal defects may lead bacterial recolonization and subsequently recurrence of the disease process following non-surgical periodontal therapy.[10] Additionally, resident bacterial strains in several anatomical structures such as tonsils or radicular dentin may lead microbial recolonization.[11,12] Use of systemic antibiotics adjunctive to SRP was suggested to obtain additional clinical benefits. Additionally, systemic antibiotics may reportedly suppress bacterial recolonization by affecting the persistent bacteria in the periodontal tissues and present an antimicrobial activity in mechanically inaccessible periodontal areas harboring periodontal pathogens.[13] Amoxicillin/metronidazole (amox/met) combination has been proposed regimen for proper antimicrobial spectrum and increased bactericidal efficacy.[14] There are various studies favoring amox/met regimen adjunctive to SRP in the treatment of periodontitis.[15,16] However, limited data has been published in accordance with bacteria recolonization and stability of treatment outcomes. However, there is limited data about the recolonization patterns of certain species in accordance with the stability of periodontal treatment outcomes.

The aim of this study was to evaluate the effect of combined amoxicillin/metronidazole treatment adjunctive to SRP on clinical periodontal parameters and subgingival *F. nucleatum*, *T. forsythia* and *P. intermedia* levels of periodontitis patients during different follow-up periods.

MATERIALS AND METHODS

Study population

The study protocol was approved by the Ethics Committee of Selcuk University, Faculty of Dentistry (2007/2-4). All subjects were informed about the study and signed informed consents were obtained at the beginning. Eligible subjects for this parallel design study were identified from a patient population attending Selcuk University, Faculty of Dentistry in Konya, Turkey. The subjects exhibiting the clinical manifestation of generalized aggressive periodontitis which was defined by the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions were evaluated whether they were appropriate for the study. Twenty-four of those patients fulfilling the inclusion criteria were participated in the study. Clinical periodontal parameters and radiological findings were considered during the selection of the volunteers for the eligibility for the study. Accordingly, inclusion criteria for the patients were to have 1) more than 20 teeth, 2) >5 mm probing depth (PD) around more than 2 teeth in each quadrant, and 3) to have attachment and bone loss around more than 3 teeth other than incisors and first molars. Exclusion criteria were as follows; smoking, allergy to amoxicillin and/or metronidazole having antibiotic therapy within the past 6 months before microbiological sampling, pregnancy, lactation, and any history of systemic condition which could influence progression and clinical characteristics of periodontal disease.

Study design and treatment protocol

Twenty-two subjects, who thoroughly concluded the study, were assigned to control (n=10) or test (n=12) group. At the first appointment, microbiological samplings performed and all clinical measurements recorded before treatment. Subjects in both groups received SRP which were conducted using ultrasonic and manual instruments. Additionally, periodontal pockets of all subjects received subgingival chlorhexidine gluconate irrigation (0.2%) at least 3 times during mechanical instrumentation. Non-surgical periodontal treatment procedures were applied to all subjects by the same clinician in two sessions within the same week. Oral hygiene instructions were given to all individuals during the treatment appointments. In test group, systemic combined amox/met (500 mg/500 mg; tid) treatment was initiated 24 hours before the treatment appointment and continued for 7 days.

Microbiological sampling and clinical measurements

The periodontal pocket which coincide the deepest radiographical bone defect in each quadrant was selected for microbial sampling. Following the isolation of sampling sites from saliva with cotton rolls, subgingival plaque was collected with a sterile curette after removal of supragingival plaque. Totally 4 subgingival plaque samples from each quadrant were pooled for each time and stored in 1 ml phosphate buffered saline at -80°C until the analysis date. Following the sampling procedure, clinical periodontal parameter measurements were recorded. Measurements included probing depth (PD), clinical attachment level (CAL), gingival index (GI) and plaque index (PI) as usual. PD and CAL of six sites of all teeth were measured with Williams® periodontal probe (Hu-Friedy, USA). Microbiological samplings and clinical measurements repeated at 1, 3 and 6 months following the active treatment phase. The patients requiring surgical treatment were scheduled for further therapeutic processes at the end of 6-month follow-up.

Microbial analysis

Bacterial DNA extraction was made using commercial kits (Qiagen DNeasy Blood & Tissue Kit, Hilden, Germany). Extracts were transferred to the tubes containing 180µl Buffer ATL and converted to suspensions. Subsequently, 20 µl Polimerase K, 200 µl Buffer AL and 200 µl 96% ethanol were added to the suspensions. Obtained PCR mix liquids were passed from QIAamp Spin colon and stored at -20°C until the PCR analysis. 100 ng DNA, 1.25 units of Taq polymerase enzyme, 5 µl PCR buffer, 5 µl 2mM dNTP and 25 pmol/µl of each primer were added in a total of 50 µl reaction volume. PCR analysis was conducted within three cycles. After cloning, DNA sequence analysis was performed using commercial Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Fostercity, USA). All used primer and probe sequences (Table 1) were purchased from Ocimum, Oligo Sysnthesis (Netherland) and stained with FAM. Real Time PCR experiments were conducted using LightCycler device (Roche, Germany).

Table 1.

Primer and probe sequences for PCR

Primer no	Primer sequence (5' → 3')	Probe sequence
F. nucleatum SENSE	CGC AGA AGG TGA AAG TCC TGT	ACT TTG CTC CCA AGT AAC ATG ATG GAA CAC GAG
F. nucleatum ANTISENSE	TGG TCC TCA CTG ATT CAC ACA	
T. forsythia SENSE	ATC CTG GCT CAG GAT GAA CG	ATG TAA CCT GCC CGC AAC AGA GGG ATA AC
T. forsythia ANTISENSE	TAC GCA TAC CCA TCC CCA A	
P. intermedia SENSE	CCA CAT ATG GCA TCT GAC GTG	ACT TGT AAG ATA GGC ATG CGT CCC ATT AGC TA
P. intermedia ANTISENSE	TCA ATC TGC ACG CTA CTT GG	

Statistical analysis

Logarithmic transformation was applied to bacteria counts before statistical analysis. The value for each bacterium was designated as zero, when the bacteria were below the detection limits. Statistical analysis was performed with SPSS for Windows 15.0 (SPSS Inc., Chicago, IL, USA). Shapiro–Wilk test was used for testing the normality of data. The data were analyzed using Paired Samples T- Test or non-parametric Wilcoxon test for intra-group comparisons. Intergroup comparisons were made via comparison of intra-group parameter differences by months. Therefore, test and control group compared by means of differences between baseline and each month. Freidman test was used for inter-group comparisons.

RESULTS

Both groups were similar with respect to demographic and periodontal properties at baseline (Table 2). Table 3 shows full-mouth clinical periodontal parameter changes in the groups between baseline and post-treatment control intervals. Alterations of periodontal parameter scores at sampling sites were also given in Table 4. All investigated clinical periodontal measurement scores were significantly decreased at 1 month comparing to baseline in both groups. Additionally, all these diminished values remained stable following post-treatment period except GI scores at sampling sites. Accordingly, there was a significant increase in only GI scores at 3rd and 6th months comparing with 1st month.

Table 5 summarizes the differences among the levels of investigated bacteria in the groups. A significant decrease were found in subgingival *F.nucleatum* counts in test group after non-surgical treatment ($p<0.01$). However, the decrease of *F. nucleatum* levels was not significant in control group during whole follow-up period. Subgingival *P. intermedia* counts significantly diminished at 1 month and almost stayed at the same levels during 3 and 6 months in both groups ($p<0.01$). In test group, there was a decrease of *T. forsythia* amounts at 1 month and a gradual increase at 3 and 6 months. Similar with test group, post-treatment increasing course of *T. forsythia* amounts was observed in control group ($p<0.01$). Furthermore, at 6 months, *T. forsythia* counts reached to baseline level in control group.

Table 2.

Demographic data of the study groups

Groups	Gender		Age
	Male	Female	
Test Group (n=12)	4	8	28.9 ± 5.7
Control Group (n=10)	4	6	27.7 ± 3.1

Data were expressed as mean ± SD

Table 6 shows the differences of the clinical periodontal parameter scores between the groups comparing with baseline. The reduction of PD and CAL scores in both groups was statistically similar at 1 month. However, the decrease in PD and CAL scores at 3 and 6 months were higher in test group comparing with control group. The difference of GI and PI scores between both groups was similar at all post-treatment months.

Table 7 demonstrates the differences of the bacteria counts between the groups comparing with baseline. The reduction of subgingival *T. forsythia* counts was significantly more in test group than control group in 1, 3 and 6 months during follow-up ($p<0.05$). *F. nucleatum* counts were insignificantly decreased in test group at 3 and 6 months comparing with control group. There was no significant difference in reduction of subgingival *P. intermedia* counts in both groups in all months. However, a slight reduction occurred in *P. intermedia* counts of control group than test group.

DISCUSSION

The aim of this study was to investigate the efficacy of amox/met treatment adjunctive to SRP during the 6 months follow-up period considering clinical parameters and recolonization pattern of some selected microorganisms. The results revealed that additional amox/met treatment exhibit favorable clinical results in periodontitis patients. It was determined that only the levels of *T. forsythia* were affected by adjunctive antibiotic regimen at each time periods when compared with controls.

Periodontal disease classification has been recently updated.[17] Accordingly, the terms “aggressive periodontitis” and “chronic periodontitis” have been abandoned. Instead of these statements, the term “periodontitis” has been accepted to identify the disease. Since we have assigned the subjects before the recent classification update, the patients who exhibit the clinical manifestations of generalized aggressive periodontitis were included to this study.[18] In accordance with new classification, we have used “periodontitis” term in this article.[17]

The efficacy of systemic antibiotic regimens adjunctive to non-surgical periodontal therapy has been subject of numerous clinical studies.[19,20] Considering the literature, major interest of the researchers was focused on amox/met supplementation. Guerrero et al (2005) reported that amox/met administration as an adjuct to to SRP in periodontitis patients resulted in more PD reduction and CAL gain.[21] Similarly, Xajigeorgiou et al (2006) also documented better clinical outcomes related with amox/met supplementation.[22] The results of some other studies conducted by Mombelli et al (2005), Ehmke et al (2005), Gomi et al (2007) and Goodson et al (2012) have also supported the clinical benefits of amox/met adjunction to SRP.[23-26] However, Lopez et al (2006) reported that amox/met supplementation to SRP did not exhibit any significant clinical benefits comparing with controls.[27] Likewise, Valera et al (2011) documented that adjunctive amox/met treatment did not improve PD and CAL levels when compared with controls.[28] Ribeiro et al (2009) reported that there was no significant difference between amox/met applied group and control subjects with respect to PD reduction.[29] The results of this study support the argument that amox/met treatment adjunctive to SRP may provide additional clinical benefits.

There is relatively limited data about the impact of adjunctive antibiotic administration on microbiological profile and recolonization pattern of the microorganisms. Considering the literature it can be seen that most of these studies have been utilized DNA hybridization based microbiological methods.[30] However, based on its high sensitivity and specifity, Real-Time PCR is an accepted microbiological approach in quantification of bacterial species.[31]

Accordingly, Ribeiro et al (2009) reported that adjunctive amox/met treatment exhibited comparable results with controls in lowering periodontal pathogens.[29] Similarly, Casarin et al (2012) also found that amox/met treatment provided same microbiological outcomes with control subjects who had received SRP during the 6 months.[32] In another study utilizing real-time PCR, Clonca et al (2010) reported that *F. nucleatum* and *T. forsythia* levels were significantly decreased in antibiotic applied group.[33] Yek et al (2010) also reported the decrease of subgingival T. forsythia which is parallel to clinical improvements during 6 months.[34] The results of our study support the findings of Yek et al (2010).

Table 3.

Time - weight interaction according to the groups

Groups	Parameter	Baseline	1. month	3. month	6. month	p-value
Test Group (n=12)	PD (mm)	5.35 ± 0.62 (b)	2.9 ± 0.68 (a)	3.15 ± 0.84 (a)	3.23 ± 0.8 (a)	<0.001
	CAL (mm)	4.43 ± 0.69 (b)	2.83 ± 0.62 (a)	2.93 ± 0.79 (a)	3.03 ± 0.81 (a)	<0.001
	GI	2.13 ± 0.38 (b)	1.36 ± 0.44 (a)	1.34 ± 0.31 (a)	1.48 ± 0.39 (a)	<0.001
	PI	2.28 ± 0.32 (b)	1.4 ± 0.39 (a)	1.35 ± 0.28 (a)	1.68 ± 0.38 (a)	<0.001
Control Group (n=10)	PD (mm)	5.18 ± 0.53 (b)	3.21 ± 0.45 (a)	3.36 ± 0.57 (a)	3.47 ± 0.61 (a)	<0.001
	CAL (mm)	4.439 ± 0.64 (b)	3. ± 0.43 (a)	3.21 ± 0.42 (a)	3.44 ± 0.51 (a)	<0.001
	GI	2.31 ± 0.27 (b)	1.18 ± 0.35 (a)	1.39 ± 0.38 (a)	1.8 ± 0.37 (a)	<0.001
	PI	2.22 ± 0.24 (b)	1.23 ± 0.38 (a)	1.32 ± 0.33 (a)	1.88 ± 0.42 (a)	<0.001

Data were expressed as mean ± SD. Significant differences were found in all parameters. The data were analyzed using Paired two sample t- test. Non-parametric Wilcoxon test was used for the analysis of GI scores. Distinct letters represent statistically significant differences in each group (p<0.05).

Table 4.

Changes of the clinical periodontal parameter scores in the groups at sampling sites

Groups	Parameter	Baseline	1. month	3. month	6. month	*p-value*
Test Group (n=12)	PD (mm)	7.25 ± 0.82 (b)	4.32 ± 0.96 (a)	4.17 ± 0.73 (a)	4.29 ± 0.54 (a)	*<0.001*
	CAL (mm)	5.25 ± 0.82 (b)	2.45 ± 0.9 (a)	2.2 ± 0.7 (a)	2.57 ± 1.14 (a)	*<0.001*
	GI	2.49 ± 0.24 (c)	1.16 ± 0.1 (a)	1.55 ± 0.3 (b)	1.63 ± 0.26 (b)	*<0.001*
	PI	2.09 ± 0.26 (b)	1.16 ± 0.1 (a)	1.81 ± 0.47 (b)	1.92 ± 0.39 (b)	*<0.001*
Control Group (n=10)	PD (mm)	6.91 ± 1.31 (b)	4.7 ± 1.03 (a)	4.82 ± 1.14 (a)	5.17 ± 0.88 (a)	*<0.001*
	CAL (mm)	5.01 ± 1.25 (b)	2.7 ± 1.03 (a)	2.82 ± 1.14 (a)	3.27 ± 0.78 (a)	*<0.001*
	GI	2.5 ± 0.19 (d)	1.14 ± 0.06 (a)	1.54 ± 0.27 (b)	1.7 ± 0.16 (c)	*<0.001*
	PI	2.04 ± 0.36 (b)	1.19 ± 0.1 (a)	1.8 ± 0.29 (b)	1.84 ± 0.09 (b)	*<0.001*

Data were expressed as mean ± SD. Significant differences were found in all parameters. The data were analyzed using Paired two sample t- test. Non-parametric Wilcoxon test was used for the analysis of GI scores. Distinct letters represent statistically significant differences in each group (p<0.05).

Table 5.

Changes of the bacteria counts in the groups

Groups	Bacteria	Baseline	1. month	3. month	6. month	*p-value*
Test Group (n=12)	*F. nucleatum*	6.39 ± 2.21 (b)	4.81 ± 1.63 (a)	3.85 ± 2.52 (a)	3.85 ± 2.11 (a)	*0.001**
	P. intermedia	5.99 ± 1.38 (b)	4.3 ± 0.92 (a)	4 ± 1.32 (a)	3.92 ± 1.59 (a)	*<0.001**
	T. forsythia	7.17 ±1 (c)	2.85 ±2.78 (a)	3.83 ± 2.93 (a,b)	5.69 ± 1.31 (b)	*0.001**
Control Group (n=10)	*F. nucleatum*	6.1 ± 2.11	4.27 ± 2	5.64 ± 1.24	5.24 ± 0.96	*0.293*
	P. intermedia	6.37 ± 2.53 (b)	3.85 ± 2.72 (a)	3.51 ± 3.22 (a)	4.6 ± 1.77 (a)	*0.008**
	T. forsythia	5.83 ± 0.69 (c)	4.05 ± 0.67 (a)	4.62 ± 0.75 (a,b)	5.83 ± 1.35 (b,c)	*0.007**

**p<0.05. Data were expressed as mean ± SD after Log transformation. The data were analyzed using Paired two sample t- test. Non-parametric Wilcoxon test was used for the analysis of T. forsythia counts. Distinct letters represent statistically significant differences in each group (p<0.05).*

Table 6.

The differences of the clinical periodontal parameter scores between the groups comparing with baseline

	Differences with respect to the months	Test Group (n=12)	Control Group (n=10)	Mean of the Differences	Standard Error	*p-value*
PD (mm)	0-1	-2.92	-2.21	0.71	0.44	*0.122*
	0-3	-3.07	-2.08	0.98	0.43	*0.035**
	0-6	-2.95	-1.73	44562	0.47	*0.018**
CAL (mm)	0-1	-2.8	-2.31	0.48	0.45	*0.297*
	0-3	-3.04	-2.18	0.85	0.41	*0.038**
	0-6	-2.67	-1.74	0.92	0.54	*0.049**
GI	0-1	-1.32	-1.35	-0.02	0.09	*0.974*
	0-3	-0.94	-0.95	-0.01	0.18	*0.771*
	0-6	-0.85	-0.8	0.05	0.15	*0.418*
PI	0-1	-0.92	-0.84	0.08	0.13	*0.547*
	0-3	-0.27	-0.23	0.03	0.23	*0.878*
	0-6	-0.16	-0.2	-0.03	0.17	*0.847*

**p<0.05. Data were expressed after Log transformation. Freidman test was used for multiple comparisons. Minus signs indicates the reduction in bacteria counts.*

Table 7.

The differences of the bacteria counts between the groups comparing with baseline

	Differences with respect to the months	Test Group (n=12)	Control Group (n=10)	Mean of the Differences	Standard Error	*p-value*
F.nucleatum	0-1	-1.82	-1.58	-0.24	43709	*0.825*
	0-3	-0.46	-2.54	43648	42005	*0.088*
	0-6	-0.85	-2.54	25204	0.86	*0.065*
P.intermedia	0-1	-2.52	-1.68	-0.83	0.97	*0.4*
	0-3	-2.86	-1.99	-0.86	43678	*0.434*
	0-6	-1.77	-2.06	0.29	0.65	*0.661*
T. forsythia	0-1	-1.78	-4.31	19391	0.8	*0.009**
	0-3	-1.21	-3.34	43801	0.87	*0.036**
	0-6	0	-1.47	17168	0.61	*0.043**

**p<0.05. Data were expressed after Log transformation. Freidman test was used for multiple comparisons. Minus signs indicates the reduction in bacteria counts.*

In the present study, systemic amox/met treatment did not exhibit an additional effect in reduction of subgingival *P. intermedia* amounts in periodontitis patients. This may be explained by high degree of antibiotic resistance pattern of *P. intermedia*.[35] Besides, *P.intermedia* is thought as reservoir for antibiotic resistance genes and potential source of other species.[36] Despite the control group, a significant reduction were found in *F. nucleatum* levels in antibiotic group. Accordingly, clinical benefits of antibiotic supplementation may be related with the suppression of *F. nucleatum* during the 6-month follow-up.

Appropriate dosage and duration issues of adjunctive amox/met therapy have been discussed in the literature. In a recent meta-analysis, McGowan et al (2017) evaluated the clinical data obtained from a number of clinical trials with respect to duration and dosage.[16] Accordingly, they have concluded that 7-day regimen of 500/500 mg or 500/400 mg of amoxicillin and metronidazole would be the most appropriate approach based on the results of previous research. In our study, antibiotic administration schedule was in accordance with the suggestion of McGowan et al (2017).[16] In this study, no additional agent was used to target antibiotic resistant species. Accordingly, additional clavulanic acid supplementation would influence the results. Lack of randomization and blinding are the limitations of this study.

Within the limitations of this study, it can be concluded that amoxicillin/metronidazole treatment adjunctive to scaling and root planing may provide additional clinical benefits and suppress *T. forsythia* recolonization.

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REFERENCES

1. Meng H, Xu L, Li Q, Han J, Zhao Y. Determinants of host susceptibility in aggressive periodontitis. Periodontol 2000 2007; 43: 133-59.
2. Marsh PD. Dental plaque: biological significance of a biofilm and community life-style. J Clin Periodontol 2005; 32: 7-15.
3. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL. Microbial complexes in subgingival plaque. J Clin Periodontol 1998; 25: 134-44.
4. Bradshaw DJ, Marsh PD, Watson GK, Allison C. Role of Fusobacterium nucleatum and coaggregation in anaerobe survival in planktonic and biofilm oral microbial communities during aeration. Infect Immun 1998; 66: 4729-32.
5. Sharma A. Virulence mechanisms of Tannerella forsythia. Periodontol 2000 2010; 54: 106-16.
6. 6. Sharma A, Inagaki S, Sigurdson W, Kuramitsu HK. Synergy between Tannerella forsythia and Fusobacterium nucleatum in biofilm formation. Oral Microbiol Immun 2005; 20: 39-42.
7. Huang RJ, Li MY, Gregory RL. Bacterial interactions in dental biofilm. Virulence 2011; 2: 435-44.
8. Mombelli A. Maintenance therapy for teeth and implants. Periodontol 2000 2019; 79: 190-9.
9. Suvan JE. Effectiveness of mechanical nonsurgical pocket therapy. Periodontol 2000 2005; 37: 48-71.
10. Mombelli A. Microbial colonization of the periodontal pocket and its significance for periodontal therapy. Periodontol 2000 2018; 76: 85-96.
11. Slots J, Ting M. Systemic antibiotics in the treatment of periodontal disease. Periodontol 2000 2002; 28: 106-76.
12. Adriaens PA, Edwards CA, De Boever JA, Loesche WJ. Ultrastructural observations on bacterial invasion in cementum and radicular dentin of periodontally diseased human teeth. J Periodontol 1988; 59: 493-503.
13. Feres M, Figueiredo LC, Soares GMS, Faveri M. Systemic antibiotics in the treatment of periodontitis. Periodontol 2000 2015; 67: 131-86.
14. Schlagenhauf U, Burgemeister S. Adjunctive use of amoxicillin/metronidazole in aggressive periodontitis refractory to mechanical/surgical therapy. Clinical results after 1 year. J Dent Res 2002; 81: B249.
15. Rabelo CC, Feres M, Goncalves C, et al. Systemic antibiotics in the treatment of aggressive periodontitis. A systematic review and a Bayesian Network meta-analysis. J Clin Periodontol 2015; 42: 647-57.
16. McGowan K, McGowan T, Ivanovski S. Optimal dose and duration of amoxicillin-plus-metronidazole as an adjunct to non-surgical periodontal therapy: A systematic review and meta-analysis of randomized, placebo-controlled trials. J Clin Periodontol 2018; 45: 56-67.
17. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. J Clin Periodontol 2018; 45: 1-8.
18. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4: 1-6.
19. Keestra JAJ, Grosjean I, Coucke W, Quirynen M, Teughels W. Non-surgical periodontal therapy with systemic antibiotics in patients with untreated aggressive periodontitis: a systematic review and meta-analysis. J Periodontal Res 2015; 50: 689-706.
20. Keestra JAJ, Grosjean I, Coucke W, Quirynen M, Teughels W. Non-surgical periodontal therapy with systemic antibiotics in patients with untreated chronic periodontitis: a systematic review and meta-analysis. J Periodontal Res 2015; 50: 294-314.
21. Guerrero A, Griffiths GS, Nibali L, et al. Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: a randomized placebo-controlled clinical trial. J Clin Periodontol 2005; 32: 1096-1107.
22. Xajigeorgiou C, Sakellari D, Slini T, Baka A, Konstantinidis A. Clinical and microbiological effects of different antimicrobials on generalized aggressive periodontitis. J Clin Periodontol 2006; 33: 254-64.
23. Mombelli A, Brochut P, Plagnat D, Casagni F, Giannopoulou C. Enamel matrix proteins and systemic antibiotics as adjuncts to non-surgical periodontal treatment: clinical effects. J Clin Periodontol 2005; 32: 225-30.
24. Ehmke B, Moter A, Beikler T, Milian E, Flemmig TF. Adjunctive antimicrobial therapy of periodontitis: Long-term effects on disease progression and oral colonization. J Periodontol 2005; 76: 749-59.
25. Gomi K, Yashima A, Nagano T, Kanazashi M, Maeda N, Arai T. Effects of full-mouth scaling and root planing in conjunction with systemically administered azithromycin. J Periodontol 2007; 78: 422-9.
26. Goodson JM, Haffajee AD, Socransky SS, et al. Control of periodontal infections: A randomized controlled trial I. The primary outcome attachment gain and pocket depth reduction at treated sites. J Clin Periodontol 2012; 39: 526-36.

27. Lopez NJ, Socransky SS, Da Silva I, Japlit MR, Haffajee AD. Effects of metronidazole plus amoxicillin as the only therapy on the microbiological and clinical parameters of untreated chronic periodontitis. J Clin Periodontol 2006; 33: 648-60.
28. Varela VM, Heller D, Silva-Senem MX, Torres MCMB, Colombo APV, Feres EJ. Systemic Antimicrobials Adjunctive to a Repeated Mechanical and Antiseptic Therapy for Aggressive Periodontitis: A 6-Month Randomized Controlled Trial. J Periodontol 2011; 82: 1121-30.
29. Ribeiro ED, Bittencourt S, Zanin ICJ, et al. Full-Mouth Ultrasonic Debridement Associated With Amoxicillin and Metronidazole in the Treatment of Severe Chronic Periodontitis. J Periodontol 2009; 80: 1254-64.
30. Paster BJ, Dewhirst FE. Molecular microbial diagnosis. Periodontol 2000 2009; 51: 38-44.
31. Boutaga K, Van Winkelhoff AJ, Vandenbroucke-Grauls CMJE, Savelkoul PHM. The additional value of real-time PCR in the quantitative detection of periodontal pathogens. J Clin Periodontol 2006; 33: 427-33.
32. Casarin RCV, Ribeiro ED, Sallum EA, Nociti FH, Goncalves RB, Casati MZ. The Combination of Amoxicillin and Metronidazole Improves Clinical and Microbiologic Results of One-Stage, Full-Mouth, Ultrasonic Debridement in Aggressive Periodontitis Treatment. J Periodontol 2012; 83: 988-98.
33. Cionca N, Giannopoulou C, Ugolotti G, Mombelli A. Microbiologic Testing and Outcomes of Full-Mouth Scaling and Root Planing With or Without Amoxicillin/Metronidazole in Chronic Periodontitis. J Periodontol 2010; 81: 15-23.
34. Yek EC, Cintan S, Topcuoglu N, Kulekci G, Issever H, Kantarci A. Efficacy of Amoxicillin and Metronidazole Combination far the Management of Generalized Aggressive Periodontitis. J Periodontol 2010; 81: 964-74.
35. Matto J, Asikainen S, Vaisanen ML, et al. Beta-lactamase production in Prevotella intermedia, Prevotella nigrescens, and Prevotella pallens genotypes and in vitro susceptibilities to selected antimicrobial agents. Antimicrob Agents Chemother 1999; 43: 2383-8.
36. Duncan MJ. Genomics of oral bacteria. Crit Rev Oral Biol M 2003; 14: 175-87.

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