



Clinical, Microbiological and Radiologic evaluation of topical use of Co-Amoxiclav 25% dental gel in the treatment of adult periodontitis

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ABSTRACT

The aim of this study was to compare the effect of the topical application of co-amoxiclav gel with placebo in the treatment of adult periodontitis by conducting clinical, microbiological and radiologic measurements. A randomized double-blind placebo-controlled clinical trial design was employed. Subjects were 12 patients with adult periodontitis divided into two control and experimental groups both initially received subgingival scaling and root planning therapy. Clinical, microbiological and radiologic examinations were carried out before treatment and on days 15, 45, and 90 after gel treatment in the experimental group. Radiographic analysis was performed using Trophy radiovisiography (RVG) system determining bone density and bone loss in the study sites. Paired-t test, Wilcoxon matched-pairs test, and binominal test were used for statistical analysis. The statistical analyses showed that both treatments were effective in reducing PPD and BOP over the 3-month period. At the end of follow-up period, the mean reduction in PPD and BOP were 1.71 mm and 54.83%, respectively. The increase in mean of alveolar bone crest density (38.16mm) was statistically significant in gel treatment ($p < 0.03$) but it was not significant in placebo group. There was a significant difference between the two treatments with respect to the reduction in proportions of anaerobic gram-negative bacilli during 0-15 and 0-90 day periods. It was concluded that the use of a topically applied co-amoxiclav 25% gel seems to be effective as a conventional mechanical therapy in the treatment of adult periodontitis.

Keywords: adult periodontitis; treatment; antibiotics; co-amoxiclav; systemic application.

INTRODUCTION

Periodontitis is a set of inflammatory diseases affecting the periodontium, i.e., the tissues that surround and support the teeth. Periodontitis involves progressive loss of the alveolar bone around the teeth, and if left untreated, can lead to the loosening and subsequent loss of teeth. Periodontitis is caused by microorganisms such as *Actinobacillus actinomycetemcomitans* that adhere to and grow on the tooth's surfaces, along with an over-aggressive immune response against these microorganisms. Many studies have been conducted to control the microbial causes of periodontal diseases using microbial agents. The use of topical antimicrobial agents in mouthwash as an adjunctive therapy for Plaque control in periodontal treatments was common for many years [1, 2]. Also, antimicrobial agents were used topically for regenerative periodontal therapy e.g. mixing antibiotics with bone graft [3, 4]. "Topical application of a drug is a form of local delivery. Topical application generally refers to delivery

of an agent to an exposed surface; for example, topical antibiotics for acne or topical antimicrobial rinses for plaque and gingivitis control” [5]. Local delivery systems for the subgingival area (e.g. subgingival irrigation) have been developed for professional application of antimicrobial agents in the dental office or home [6, 7]. One of these systems is controlled-release local delivery systems in which the antimicrobial is available at therapeutic levels for several days [5]. Most local delivery reports in the periodontal literature have involved tetracycline, metronidazole, or chlorhexidine, e.g. [8-11]. Haffajee et al. [12] found that adjunctive systemically administered agents including Augmentin, tetracycline, ibuprofen or a placebo can increase the treatment of periodontal infections. Some of these antibiotic agents have been used for the treatment of adult periodontitis, e.g. [13, 14]. Lasers are increasingly being used in treatments for chronic adult periodontitis; however “No consistent evidence supports the efficacy of laser treatment as an adjunct to non-surgical periodontal treatment in adults with chronic periodontitis” [15].

There are some studies that have evaluated the effect of amoxicillin/clavulanic acid or co-amoxiclav on the treatment of periodontitis, e.g. [16]. It is a combination antibiotic consisting of amoxicillin trihydrate, a β -lactam antibiotic, and clavulanate potassium, a β -lactamase inhibitor. This combination results in an antibiotic with an increased spectrum of action and restored efficacy against amoxicillin-resistant bacteria that produce β -lactamase. Since periodontitis is one of the most common chronic diseases of adults, and the systemic administration of co-amoxiclav is effective for treatment of the patients with different types of periodontal diseases, in this study we attempted to investigate topically applied co-amoxiclav in the treatment of adult periodontitis. Our purpose is to compare the effects of co-amoxiclav 25% gel plus subgingival scaling and root planning therapy with placebo technique by measuring following parameters: probing pocket depth, bleeding on probing, bone level, alveolar bone crest density, and proportions of pigmented anaerobic and aerobic gram-negative and gram-positive rods, cocci and bacilli. The trial period was three months.

MATERIALS AND METHODS

Subjects

This study is a randomized double-blind placebo-controlled clinical trial. Our purpose is to conduct clinical, radiographic, microbiological measurements on co-amoxiclav gel to investigate the effects of its topical use on treatment of adult periodontitis. Subjects were those 12 persons with adult periodontitis visited the Department of Periodontics at the School of Dentistry, Tehran University of Medical Science in Tehran, Iran who were included in the study if they had periodontal probing pocket depth of 5 mm or more on the mandibular molar. Exclusion criteria were: having any systemic disease, any periodontal treatment in the last six months, and any antibiotics use in the last six months; pregnancy and lactation; having occlusal trauma and any orthodontic treatment; smoking, poor oral health, and loss of one or both mandibular first and second molars.

Preparation of co-amoxiclav 25% dental gel

To prepare the gel of co-amoxiclav with 25% concentration (This percent was determined based on its therapeutic dosage which is about 375 mg, four times per day), first, 0.5% of polycarbophil powder (Noveon® AA1, BF Goodrich) was mixed with glycerin; the gel was formed after 24 h. Next, the co-amoxiclav powder containing an inert material was ground into fine powder using mortar and then, polyethylene glycol PEG800 and glycerin were added. Finally, the prepared gel was added to the above mixture (i.e. PEG800, glycerin, and co-amoxiclav powder) and its PH was adjusted with triethanolamine.

Clinical parameters

After giving necessary explanations about the methodology to the patients and obtaining the informed consent from them, the Probing Pocket Depth (PPD) and Bleeding on Probing (BOP) tendency to control the inflammatory state were assessed at mid-buccal, mid-mesial, mid-distal and mid-lingual sites of the studied tooth (mandibular left and right molars). To measure PPD, a probe (Hu-Friedy Williams) was used. Also, to measure BOP, if the bleeding occurs within ten seconds after initial probing, BOP is considered positive; otherwise, it is considered as negative [17]. All the measurements were made before the treatment and on 15, 45 and 90 days after the second application of co-amoxiclav gel.

Radiographic evaluation

Trophy Radiovisiography (RVG) system was used for preparing X-rays images. We also assessed occlusion rate from both mandibular left and right molars to stabilize the repetition of radiography paralleling technique. To do this, when X-ray Film Holder was fixed to the patient's teeth by means of RAPID impression material, a part of the

material was placed on the occlusal surface of the mandibular molars and then was kept in the refrigerator after radiography (as described in [18]). It should be noted that the type of device and radiation time were the same for all the patients. Although radiographies do not display the Buccal and lingual bone morphology, they provide useful information about bone level in the interdental area [19]. It should be noted that dental occlusal surface of all the patients 90 days after the treatment went under radiographic evaluation using the Stent. In the radiographies, the alveolar bone densities as well as bone loss in the distance from Cemento-Enamel Junction to the alveolar bone crest were reexamined (see Fig. 1 and 2).

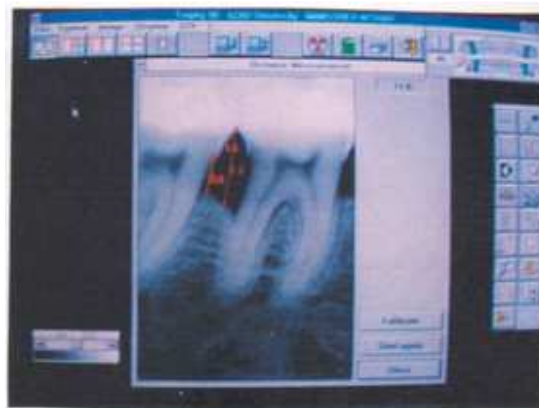


Figure 1. Alveolar bone loss measurement using RVG

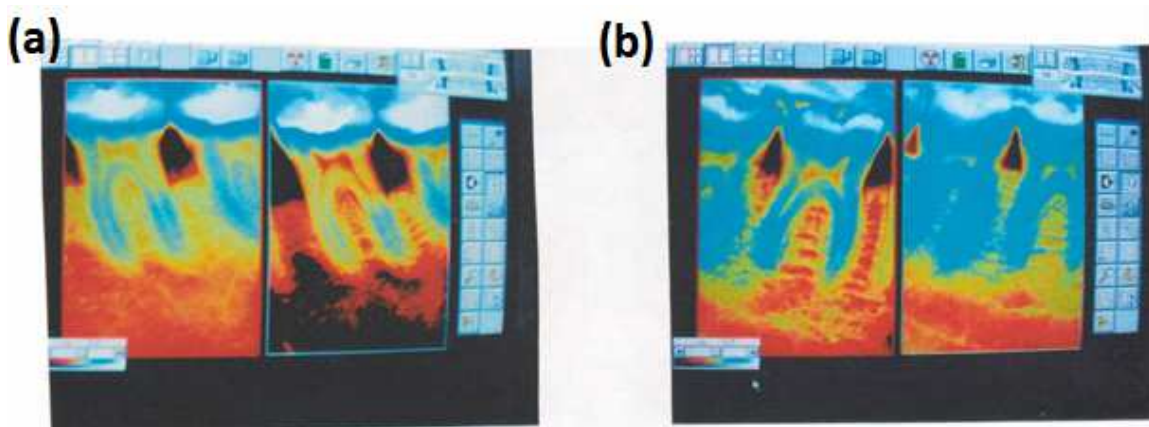


Figure 2. Alveolar bone density measurement using RVG before and after gel treatment; (a) experimental group, (b) control group

Microbiological parameters

Microbiological samples were collected from the base of the periodontal pocket from patients. They were obtained as follows: after determining the location of sampling, the surface of gingival margin was cleaned and dried by sterile gauze. Then, the sites were completely isolated with cotton rolls. Afterwards, using a sterile curette, the supragingival plaque was completely removed from the tooth surface, and then the tooth was air dried. Samples were taken with a curette which was inserted into the pocket until resistance was met and kept for 10 seconds. After removal, it was quickly entered into vials containing 3cc of thioglycollate solution without dextrose (transport medium). The samples then were transferred to the chamber in less than 30 minutes. Samples were homogenized for 30 seconds. The transport solution then was inoculated by use of anesthetic needle with Blood Agar (containing 5% sheep blood) and Brucella Agar (containing Vitamin K and Hemin) media, and dedicated medium of *Actinobacillus actinomycetemcomitans*, TSBV agar (containing tryptic soy agar, yeast extract, horse serum, bacitracin, and vancomycin). So, for each patient in each time six plates were cultured. All the plates of aerobic and anaerobic cultures were incubated at 37°C for 48-72 h. Anaerobic bacteria were recultured and went under aerobic conditions and their aerotolerance was tested. Gram-positive and gram-negative cocci and bacilli were

revealed by using the gram stain. TSBV plates were incubated at 37°C for 72 h, and colonies of *A. actinomycetemcomitans* were identified on TSBV medium based on their star like inner structure, positive catalase reaction, preparation of a slide culture and observation of negative-gram coccobacilli. Then the number of *A. actinomycetemcomitans* colonies on TSBV medium was numerated, and as a result the total number of colonies peronecc of the sample was counted by multiplying the number of colonies on TSBV plate by (2×10^3) dilution factor or loop factor. If the minimum number of counted colonies be higher than 10^3 , *A. actinomycetemcomitans* is positive. All the microbiologic measurements were repeated on 15 and 90 days after the treatment.

Periodontal scaling and root planning therapy

Full-mouth supragingival and subgingival scaling was performed using ultrasonic device for all the patients the same in three sessions. The used gels which were formulated under aseptic conditions were supplied to the clinic in two cartridges coded 1 and 2, one had co-amoxiclav gel and other with placebo gel. Neither doctor nor the patients were aware of their content. In subgingival Scaling and Root Planning (SC/RP) therapy, the co-amoxiclav and placebo gels were placed randomly into the pockets of mandibular left and right molars using disposable insulin syringe. The pockets were completely filled such that the dental gel could be observed at the gingival margin [20]. The sites were fully covered with the Co-Pack. The patient was not allowed to drink or eat two hours after gel administration, and at the time of gel application, the patient had to have a soft diet. On day 15, individual oral hygiene instructions were given to all participants. Then, once every 15 days until the end of follow-up period, the polishing was repeated using prophylaxis paste.

Statistical analysis

Differences in the clinical and radiographic parameters between control and experimental groups were tested by Paired T test. Significance of microbiological parameters were tested by binominal test, and the difference between patients with respect to the number of *A. actinomycetemcomitans* samples were analyzed using Wilcoxon matched-pairs test.

RESULTS

Clinical tests

A total of 12 patients with no previous periodontal treatment (7 men and 5 women) were entered into the study. The age range was 21 to 60 years. A total of 60 teeth were treated in the trial. Of these, 30 teeth were randomly included in group A with co-amoxiclav gel (experimental group), and 30 teeth were included in group B with placebo gel (control group).

The changes in clinical parameters including PDD and BOP before the treatment (day=0), and on 15, 45 and 90 days after the treatment within the experimental and control groups were compared using paired T-test. We found that on 90 days after the treatment with the gel, the mean reduction of PPD was 3.04 mm for the experimental group and 1.71 mm for the control group, and both treatments significantly reduced PPD ($p < 0.004$) (see Fig. 3). The mean reduction of PPD from day 15 to day 90 after treatment with the gel for the experimental group was statistically significant ($p < 0.007$), but for the control group it was not ($p = 0.15$). From day 45 to day 90, the mean reduction of PPD for the experimental group was also statistically significant ($p < 0.004$), and for the control group it was not ($p = 0.36$).

Moreover, the mean reduction of BOP on 90 days after the treatment with the gel, were 73 and 54.83% for the experimental and control groups, respectively, so it indicates that both treatments significantly lead to BOP reduction ($p < 0.005$) (see Fig. 4). For day 15 to day 90 after treatment, mean reduction of BOP was significant for the experimental group ($p < 0.007$) but it was not significant for the control group ($p = 0.14$). Similarly, from day 45 to 90 days after treatment, the mean reduction of BOP was not significant for the experimental group ($p = 0.16$) but it was significant for the control group ($p < 0.08$).

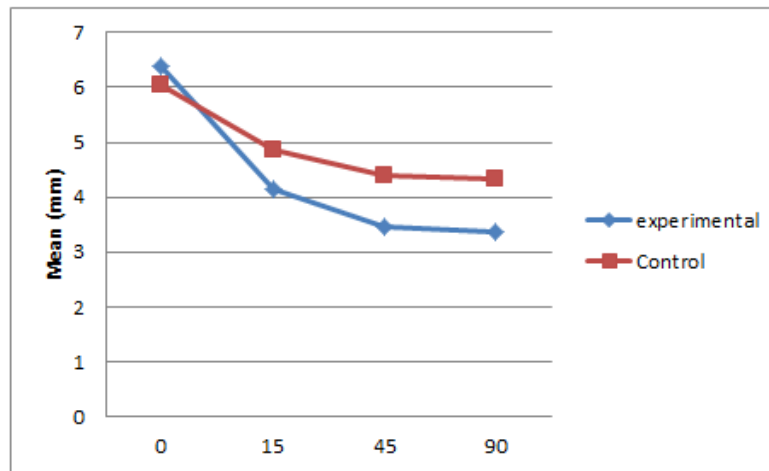


Figure 3. Mean reduction of PPD before treatment (day=0), and on 15, 45 and 90 days after treatment with the co-amoxiclav gel in control and experimental groups

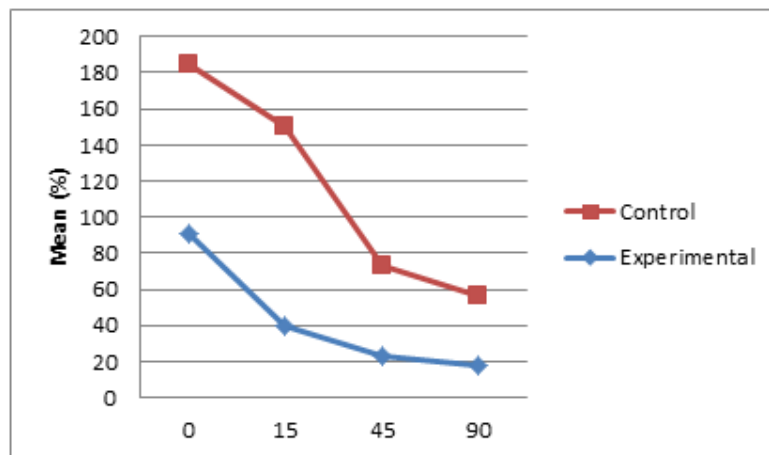


Figure 4. Mean reduction of BOP before treatment (day=0), and on 15, 45 and 90 days after treatment with the co-amoxiclav gel in control and experimental groups

Radiographic analysis

The changes in radiographic parameters including bone loss at the distance between cemento-enamel junction and the alveolar bone crest (CEJ-ABC) for the first molar (BL_6) and second molar (BL_7), at the distance from the contact point in teeth 6 and 7 to ABC (BL_{cp}), as well as the density of ABC in the interdental area of first and second molar (BD) before (day 0) and at the end of the follow-up period (day 90) within the experimental and control groups were compared using paired T-test (see Fig. 5). Results showed that the mean reduction of BL_7 was 0.16 and 0.35 mm for the experimental and control groups, respectively, and it was not statistically significant for experimental group ($p=0.54$) and control group ($p=0.17$). For BL_6 , the mean reduction in the experimental and control groups were 1.01 and 0.43 mm, respectively and it was significant in the experimental group ($p<0.008$) but not in the control group ($p=0.17$). For BL_{cp} , the mean reduction for the experimental and control groups were 0.8 and 0.16 mm, respectively. This was significant in the experimental group ($p<0.03$) but not in the control group ($p=0.95$). The mean of increase in bone density (BD) of ABC was 38.16 mm for the experimental group and 10.25 mm for the control group. Paired-t test showed that this increase of bone density was significant in the experimental group ($p<0.003$) but not in placebo group (see Fig. 6).

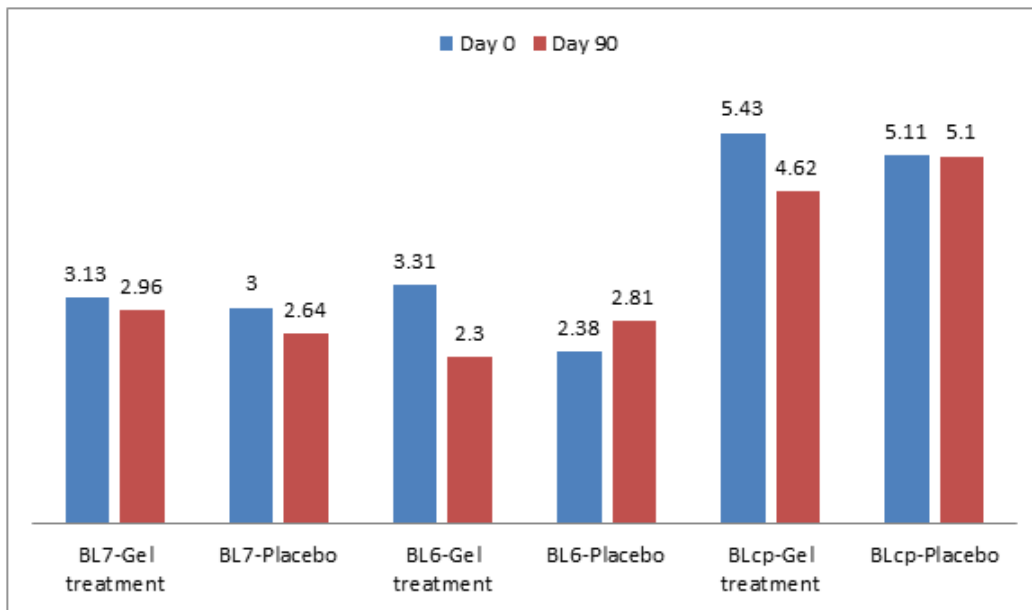


Figure 5. Mean of BL₆, BL₇ and BL_{cp} before (day=0) and 90 days after treatment with the co-amoxiclav gel in control and experimental groups

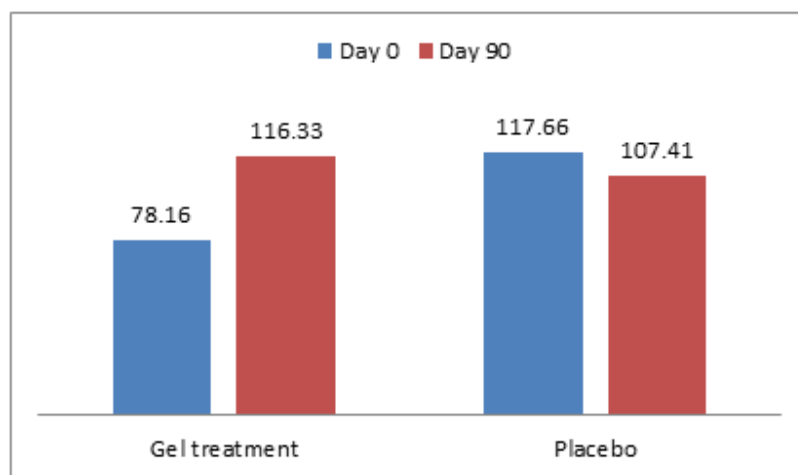


Figure 6. Density of alveolar bone crest before (day=0) and 90 days after treatment with the co-amoxiclav gel in control and experimental groups

Microbiological tests

Since amoxicillin is a semi-synthetic penicillin which contains a wide range of gram-positive and gram-negative bacteria; therefore, the total count of cultivatable bacteria both aerobic (gram-positive cocci, gram-positive bacillus, gram-negative cocci, and gram-negative bacillus), and anaerobic (gram-positive cocci, gram-positive bacillus, gram-negative cocci, and gram-negative bacillus) in sites during the observation period were compared between the experimental and control group using Binomial test. According to the results shown in tables 1 and 2, we found that:

- In both experimental and control groups, reduction of aerobic gram-positive cocci from day zero to 15, and from day zero to 90 days after treatment with the co-amoxiclav gel was significant ($p < 0.004$ and $p < 0.006$). No statistically significant differences between the treatments were found with respect to *aerobic gram-positive cocci* reduction.
- In both experimental and control groups, reduction of aerobic gram-positive bacillus was not significant during 0-15 and 0-90 days after gel treatment. The difference between the two treatments also was not significant with respect to *aerobic gram-positive bacillus* reduction.

- In the experimental group, reduction of aerobic gram-negative cocci from day zero to 15 days after the treatment was significant ($p < 0.03$), but from day zero to 90 days after the treatment it was not significant. In the control group, the difference was not statistically significant at both time intervals. Also, no statistically significant differences between the two methods were found in term of *aerobic gram-negative cocci* reduction.
- In the experimental group, reduction of aerobic gram-negative bacillus was significant during 0-15 days after the treatment ($p < 0.05$) but it was not significant during 0-90 days after the treatment. In the control group, the difference was not statistically significant at both time intervals. Moreover, with respect to *aerobic gram-negative bacillus* reduction, no significant difference was observed between the two treatments.
- In the experimental group, reduction of anaerobic gram-positive cocci was not significant during 0-15 and 0-90 days after the treatment. In the control group, this difference was not significant during 0-15 days after gel treatment, but from zero to 90 days after the treatment, a significant difference was found ($p < 0.05$). The difference between the two methods was not significant with respect to *anaerobic gram-positive cocci* reduction.
- In the experimental group, reduction of anaerobic gram-positive bacillus was significant at the period of 0-15 days after the treatment ($p < 0.05$) but during 0-90 day period, it was not significant. Similarly, in the control group, the difference was significant during 0-15 day period ($p < 0.03$) but not significant during 0-90 days after gel treatment. There was not a significant difference between the treatments with respect to *anaerobic gram-positive bacillus* reduction.
- In both groups, reduction of anaerobic gram-negative cocci was not significant during 0-15 and 0-90 daytime intervals. Furthermore, there was not a significant difference between the treatments with respect to *anaerobic gram-negative cocci* reduction.
- In both groups, reduction of anaerobic gram-negative bacillus was significant during 0-15 and 0-90 days after gel treatment ($p < 0.0006$ and $p < 0.008$). no significant difference was observed between the two treatments with respect to *anaerobic gram-negative bacillus* reduction.

Table 1. Statistical significance of difference in aerobically and anaerobically cultivable bacteria before and 15 days after the gel treatment

Cultivated bacteria	Aerobic			Anaerobic		
	Experimental group	Control group	Difference between the treatments	Experimental group	Control group	Difference between the treatments
Gram-positive cocci	$p < 0.004$	$p < 0.006$	NS	NS	NS	NS
Gram-positive bacillus	NS	NS	NS	$p < 0.05$	NS	NS
Gram-negative cocci	$p < 0.03$	NS	NS	NS	NS	NS
Gram-negative bacillus	$p < 0.05$	NS	NS	$p < 0.006$	$p < 0.006$	$p < 0.02$

Table 2. Statistical significance of difference in aerobically and anaerobically cultivable bacteria before and 90 days after the gel treatment

Cultivated bacteria	Aerobic			Anaerobic		
	Experimental group	Control group	Difference between the treatments	Experimental group	Control group	Difference between the treatments
Gram-positive cocci	$p < 0.004$	$p < 0.006$	NS	NS	$P < 0.05$	NS
Gram-positive bacillus	NS	NS	NS	NS	NS	NS
Gram-negative cocci	$p < 0.03$	NS	NS	NS	NS	NS
Gram-negative bacillus	$p < 0.05$	NS	NS	$p < 0.008$	$p < 0.006$	$p < 0.02$

Frequency of *A. actinomycetemcomitans* (Aa) samples (positive and negative) per patient detected during 0-15 and 0-90 day periods are shown in tables 3 and 4. Before gel treatment and at the end of follow-up period is shown. In the control group, before gel treatment, the frequency of positive Aa samples per patient was 6 with one negative Aa sample. After treatment there was only one patient with positive Aa and four subjects with negative Aa (Total=7). In the experimental group before gel treatment the frequency of positive Aa was 6 with no negative Aa sample. After treatment, we found two patients with positive Aa and four subjects with negative Aa sample (see table 4). Wilcoxon

matched-pairs test results showed that the difference between the treatments with respect to the number of patients with Aa+ was significant only at time interval of 0-15 days after gel treatment ($p < 0.03$).

Tables 3. Frequency of detection of *A. actinomycetemcomitans* per patient before and 15 days after gel treatment

Groups	Period	Aa+	Aa-	Total
Control group*	Before treatment	5	2	7
	After treatment	2	3	5
	Total	7	5	12
Experimental**	Before treatment	3	0	3
	After treatment	5	4	9
	Total	8	4	12

Note: * $p=0.688$, ** $p < 0.03$

Tables 4. Frequency of detection of *A. actinomycetemcomitans* per patient before and 90 days after gel treatment

Groups	Period	Aa+	Aa-	Total
Control group*	Before treatment	6	1	7
	After treatment	1	4	5
	Total	7	5	12
Experimental**	Before treatment	6	0	6
	After treatment	2	4	6
	Total	8	4	12

Note: * $p=0.75$, ** $p < 0.25$

DISCUSSION

The aim of this study was to compare the clinical, microbiological and radiographic effects of topical application of co-amoxiclav 25% gel on adult periodontitis after scaling and root planning therapy. The patients also received oral hygiene instruction on day 15 which itself can reduce gingival inflammation and result in changes in the subgingival microflora; however, Lavanchy et al. [21] showed that professional oral hygiene procedures do not influence the subgingival microbiota following scaling. So while self-performed oral hygiene procedures can cause clinical improvements, but it is unlikely that they can influence the microbiological findings.

Both treatments led to a statistically significant reduction in PPD and BOP. This reduction was persisted at 90-day follow-up period. During this period there was no reversion to the pre-treatment clinical status, and there was a significant difference between the treatments with respect to mean reduction in PPD and BOP. Reduction in probing pocket depth after scaling and root planning therapy (1.71mm in pockets with a 6.05 mm PPD) is accordance with the results of Rams & Keyes [22] and Badersten et al. [23]. They reported report a 1.4-1.5 mm reduction in pockets with a PPD of 4-6 mm.

The method employed in this study for determination of the absolute number of bacteria in a periodontal pocket is based on the assumption that the curette used for the collection of subgingival plaque samples absorbs a reproducible amount of material from the pocket [17]. It disclosed a significant reduction of the total mass of bacteria particularly after gel treatment.

In this study, in both groups, the reduction in the proportions of anaerobic gram-negative bacilli 0-15 and 0-90 days after gel treatment was significant. In addition, there was a significant difference between the treatments with respect to anaerobic gram-negative bacilli proportion reduction at both time intervals. Since obligate anaerobic microorganisms are associated with destructive periodontal diseases, co-amoxiclav has excellent activity against these subgingival bacteria. *A. actinomycetemcomitans* is a facultatively anaerobic organism and hence is not likely to be affected by changes in oxygen tension in the subgingival compartment [17]. On this basis, this study confirmed that mechanical debridement alone is unable to eliminate from subgingival plaque [24-26].

Our study showed that the difference in the number of patients with respect to the observation of Aa+ was significant only in the experimental group during 0-15 days after treatment. Furthermore, the difference between the two groups with respect to the number of patients with Aa+ was significant only at time interval of 0-15 days. It should be noted that total bacterial load was reduced using both treatments.

The results of this study revealed the effect of co-amoxiclav gel on the bone loss. The average increase in the density of alveolar bone crest was significant in the experimental group, but it was not significant in the control group. Moreover, there the difference between the treatments was significant with respect to the increase in density of alveolar bone crest. About other factors which did not show a significant difference (BL₆ and BL₇) may be the reason is the number of patients was low (the sample size was determined based on the changes in clinical parameters) or perhaps a 3-month follow-up period is too short to assess the bone changes.

CONCLUSION

In this study, co-amoxiclav 25% dental gel which has a slow release regime, was used as a therapeutic method plus SC/RP and its topical use results was compared to placebo technique. Reduction in PPD, BOP, and proportions of gram-negative bacilli as well as the increase in density of alveolar bone crest in the experimental group (SC/RP + co-amoxiclav gel) were significantly better than those in the control group (SC/RP + placebo gel). Therefore, co-amoxiclav gel can be used as an adjunctive therapy to subgingival debridement among the patients with adult periodontitis. Certainly, it is necessary to conduct further research on the effect of co-amoxiclav gel particularly on microbiological and clinical parameters of other periodontal diseases.

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