The Effects of Systemic and Locally Azithromycin Adjunct to Scaling and Root Planing on Clinical and Microbiological Periodontal Indices in Moderate to Severe Chronic Periodontitis

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Abstract: Background: Adjunctive antibiotic therapy has been proposed as a complementary treatment for periodontal disease. The aim of this randomized, double-blind, placebo-controlled clinical trial was to investigate the efficacy of the adjunctive use of local and systemic Azithromycin (AZM) in the treatment of chronic periodontitis (CP).

Methods: 120 patients with moderate to severe CP were randomly assigned in this study. For all patients, scaling and root planing (SRP) were initially performed. After one month, patients are randomly divided into four groups. Group 1: 1% AZM gel; group 2: 250 mg AZM twice daily (bid) for 3 days; group 3: placebo gel and group 4: Placebo capsules bid for 3 days. Clinical parameters included Pocket depth, Clinical attachment level, Papillary bleeding index, and Modified gingivitis index.

Result: In all groups, Parameters studied 4 months after SRP, are significantly decreased compared to baseline. The adjunctive use of local 1% AZM resulted in significant improvement in both clinical and microbiologic parameters in comparison with other groups (P < 0/05). Also, in all clinical parameters greater improvement was observed in the test groups as compared to control groups at 4 months after treatment (P < 0/05).

Conclusion: Although treatment strategies in all four groups seemed to benefit the patients, the adjunctive use of local and systemic AZM combined with SRP demonstrated significant improvement in patients with CP.

Keywords: Antibiotics, Azithromycin, Periodontitis, Scaling and Root Planning.

1. Introduction

Chronic periodontitis (CP) is an infectious disease resulting in inflammation within the tooth-supporting tissues. This chronic inflammation causes progressive attachment loss and destruction of alveolar bone [1]. Based on “specific plaque hypothesis” (Loesche 1979) losing attachments and the bone corrosion is associated with increasing the ratio of gram-negative organisms in the plaque [2-3]. Periodontal therapies can be categorized into three groups: mechanical manipulation of the subgingival environment consisting of scaling and root planing (SRP) or use of Er: YAG laser for SRP; the use of antibiotics and antiseptics; and attempt to change the environment of the microorganisms [4-7]. Although SRP is considered the “gold standard”, but there is a high recurrence rate [8]. This may be attributed to depth of pocket and presence of inaccessible sites that lead to inadequate debridement and re-establishment of a pathogenic subgingival microflora[9]. Additionally, the presence and persistence of putative periodontal pathogens such as Aggregatibacter actinomycetemcomitans after most conventional treatment tends to impair the clinical outcome [10].

Hence, chemotherapeutic approaches as adjunct to mechanical therapy might be proposed [11-12]. Generally, chemotherapeutic agents can be used in three forms: systemic antibiotic therapy, topical application of antiseptics, and local drug delivery. Among the different antimicrobials available, tetracycline group (doxycycline), penicillin, metronidazole-amoxicillin combination, fluoroquinolones group, and macrolides have been used for periodontal treatment[13-15]. Adverse effects including induction of bacterial resistant strains and hypersensitivity reactions represent a serious limit to the use of penicillin and tetracycline derivatives[16].
Macrolide antibiotics are sustained well and may be a good alternative for the treatment of odontogenic infections[17]. Azithromycin (AZM), first synthesized in 1980, represents the prototype of a novel class of macrolides named azalides. AZM, like all Macrolides works in a bacteriostatic fashion, prevents bacteria from growing by interfering with their ability to make proteins. It acts by binding to the 50s component of the bacterial ribosome, thus inhibiting translation of mRNA and interfering with microbial protein synthesis. AZM has enhanced macromolide potency and a wide antimicrobial spectrum with in vitro activity toward aerobic and anaerobic bacteria as well as gram-negative bacilli [17-20]. It is effective against periodontopathogens like Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis [20-24]. This property supports its use in the treatment of periodontal infections [25-32]. Different studies have reported the effects of administering AZM in the treatment of systemic, intraoral, and facial infections [24-33]. In addition, AZM has a long half-life in human serum and periodontal tissues [22-37]. Long half-life, simple and short dosage regime lead to improved patient compliance [29-56]. Unlike other antibiotics, AZM is found in high concentrations in fibroblasts and acute reactant cells, like polymorphonuclear leukocytes, monocytes, and lymphocytes [39-40]. The drug subsequently is transported by chemotactic mechanisms to the site where inflammation occurs[41].

Moreover, AZM has important properties that have underpinned its use in the treatment and resolution of periodontal diseases: suppressing periodontal pathogens, anti-inflammatory activity and healing through persistence at low levels in macrophages and fibroblasts in periodontal tissues[42]. Additionally, AZM has significantly less bacterial resistance to subgingival microflora of CP compared to other commonly prescribed oral antibiotics[43].

Various studies have evaluated the clinical and microbiological effects of systemic AZM as an adjunct to SRP in the treatment of periodontitis [24, 25, 27, 29, 43].

Nonetheless, the repeated and long-term systemic antibiotic therapy is fraught with drawbacks, including superimposed infections, development of resistant bacteria, hypersensitivity reaction and organ toxicity [8, 45].

Side effects of systemic AZM are rare and usually minor, and they include gastrointestinal problems, such as diarrhea, nausea, abdominal pain, and vomiting [29].

To overcome some of described limitations, local drug delivery system has been proposed. Many antimicrobials can be used in local drug delivery forms as tetracycline fibers, metronidazole gel, minocycline ointment and microspheres, chlorhexidine chip, and doxycycline hyclate in bioabsorbable polymer. In the last years, different studies have reported the clinical and microbiologic effectiveness of subgingival delivery of AZM gel [31, 38, 47, 48, 49]. Keeping the above facts in mind, the aim of this double-masked, randomized, placebo-controlled clinical trial was to compare the clinical and microbiological effects of systemic and locally delivered 1% AZM or placebo with non-surgical periodontal therapy in the treatment of CP patients harbouring A. actinomycetemcomitans and P. gingivalis.

2. Materials and Methods

Patient population A microbiological screening to detect P. gingivalis and A. actinomycetemcomitans-positive patients was performed in moderate-severe CP patients, seeking for periodontal treatment at the Department of Periodontology, Faculty of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran. The screening period lasted from May 2012 to November 2012. Patients had to fulfill the following inclusion criteria: 1) Older than 18 years, 2) At least twenty teeth, 3) Untreated moderate-severe CP with radiographic evidence of generalized alveolar bone loss > 30%, 4) Presence of at least one pocket with probing depth (PD) > 5 mm per quadrant with papillary bleeding index (PBI), 5) Detection of P. gingivalis and A. actinomycetemcomitans in subgingival samples taken at the screening visit and processed by quantitative real-time polymerase chain reaction (QRT-PCR). Exclusion criteria were: 1) Patients with history of allergy to the macrolide antibiotics, 2) Patients who were suffering from any known immunodeficiency disorders like HIV infection and systemic diseases that affect periodontal conditions such as: diabetes, blood disorders, 3) Patients who had received any surgical or non-surgical therapy 1 year prior to the start of the study, 4) Severe periodontitis with more than one tooth with a site with PD > 7 mm per quadrant except if it was scheduled for extraction, 5) Necrotizing periodontal diseases, 6) Antibiotic intake within the 4 months previous to the screening visit, 7) The lack of patient cooperation, 8) Tobacco users and alcoholics, 9) Pregnant and lactating females, 10) Use of non-steroidal anti-inflammatory drugs, 11) Patients treated with drugs such as: Anti-acid, Warfarin and Cyclosporine. Following subject selection, 30 patients were randomly recruited to one of four treatment groups.

Sample Size Calculation

Based on preliminary analyses, it was estimated that 30 subjects per group to be necessary to provide 80% power, with a significance level of 5% to detect a difference of 1 mm between the four groups mean PD reduction.
Study Design

The study was carried out as a single-center, double-masked, randomized, placebo-controlled clinical trial comparing the clinical and microbiologic efficacy of adjunctive systemic and locally delivered 1% AZM or placebo following SRP in the treatment of CP. To ensure adequate blinding, the examiner was blinded to the study group, and a separate clinician administered all treatments. This study was conducted from December 2012 to April 2013. One hundred twenty patients, 20-55 years of age, consisting of 57 males and 63 females, participated in this study. This study included four groups characterized as two test groups: SRP plus local delivery of 1% AZM (group 1) or SRP combined with systemically administered AZM at the dosage of 250 mg twice daily (bid) for 3 days (group 2), and two control groups: SRP plus local delivery of placebo gel (group 3) or SRP plus placebo capsules bid for 3 days (group 4).

Study Visits

- Screening Visit
  A full-mouth periodontal evaluation was performed in order to assess the inclusion-exclusion criteria and to select the sampling sites. Before participation, the purpose and procedures were fully explained to all patients. Patients fulfilling the inclusion criteria gave written informed consent in accordance with the Declaration of Helsinki. Patients were informed that participation would only occur if they were positive for P. gingivalis and A. actinomycetemcomitans. The microbiological sample of the screening visit was considered as baseline sample.

- Baseline Visit
  All clinical outcome variables were recorded using the Color-Coded, Double-End Williams Probe (Nordent) by one single trained and calibrated investigator. A baseline visit was scheduled and the following clinical parameters were assessed:
  - PD: To standardize assessments, PD in millimeters in single rooted teeth at six aspects (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, and mesio-lingual) calculated.
  - Clinical attachment level (CAL): As the sum of PD and gingival margin (GM) in millimeters in single rooted teeth at six aspects assessed.
  - Modified gingival index (MGI) [50]. Relied on a visual assessment of gingival changes to measure the severity of inflammation and classified into five categories.
  - PBI*: Based on sweeping a probe in the sulcus from the line angle to the interproximal contact. The intensity of any bleeding classified into five categories.

Participants, by means of a draw, were randomized to one of four experimental groups.
1. SRP plus local delivery of 1% AZM (Group 1);
2. SRP combined with systemically administered AZM capsules at the dosage of 250 mg twice daily (bid) for 3 days (Group 2);
3. SRP plus local delivery of placebo gel (Group 3);
4. SRP plus placebo capsules bid for 3 days (Group 4).

The randomization list was kept by another author until the statistical analyses were performed.

- Treatment Visits
  The subjects received a two-phase treatment. Phase 1 consisted of SRP and oral hygiene instructions. Phase 2 include the medications. Within 3 days from baseline, full-mouth SRP was carried out using an ultrasonic device (Mectron, Carasco, GE, Italy) and a manual periodontal curette (Hu-Frieday, Chicago, IL, USA) in two sequential visits, during 5 days. SRP was performed by another calibrated and experienced periodontist. No antiplaque agent was prescribed after SRP. All patients received standardized oral hygiene instruction (modified bass brushing technique). During the treatment sessions, oral hygiene was assessed and home care instructions were reinforced. One month after the last SRP session, clinical parameters included PD, CAL, MGI, and PBI were recorded. Thereafter, the adjunctive medications were prescribed by another periodontist who was unaware of the clinical recordings.

Follow-up Protocol

One month after SRP (before medications), the first follow-up visit was appointed. New follow-up visits 1, 2, and 3 months after medications (2, 3, and 4 months after SRP) were scheduled. Clinical parameters at these
sessions were assessed. The subgingival plaque samples for microbiologic assessment (P. gingivalis and A. actinomycetemcomitans counts) were taken at baseline and at the 4-month visit after SRP.

**Microbiological Samples**

Using qRT-PCR and species-specific primers for two periodontopathic bacteria (P. gingivalis and A. actinomycetemcomitans), microbiologic assessment was done for 10 patients from each group at baseline and at 4 months after SRP (totally 80 samples). From each patient, sample was taken from the mesio-buccal surface of a single-rooted tooth with the deepest PD. After removal of supragingival soft deposits, subgingival biofilm samples were collected using sterile curettes and standardized #30 sterile paper points. Area-specific Gracey curettes were carefully inserted through the pocket orifice as far apically as possible. Before sampling, the sites were isolated by applying cotton rolls and dried by an air syringe. The paper points were kept in place for 30 seconds. Paper points contaminated with blood were discarded. The paper points and samples with the patients' details on them were placed into microtubes containing phosphate-buffered solution (PBS) and stored in -20°C until the qRT-PCR procedure was performed. Genomic DNA was extracted using an Extract 5 prime kit. qRT-PCR was performed with a Real Time PCR StepOne system.

**Preparation of Medications**

The placebo and AZM capsules were provided by Exir Pharmaceutical Company. Subjects in the test group received a package containing six AZM 250 mg coated capsules (Zimexir, Exir, Boroojerd, Iran). The control group received identical package containing six placebo capsules (containing inactive ingredients). Test and control capsules were identical in color, form, size, and weight. The patients were instructed to take two capsules bid for 3 consecutive days at same moment of the day (1 hour before or 2 hours after meals). The dentist called each patient during the next 3 days to memorialize to take the capsules. 1% AZM gel was prepared at Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, n. The AZM gel was prepared by 2% AZM in situ gel (Avindo Gel, CFL Pharmaceuticals Ltd, Panjim India). Briefly, an accurately weighed amount of 1% Carbomer gel (974P) was placed in a glass vial. A weighed amount of 2% AZM gel with the same proportion (with a ratio of 1:1) was added to the above gel and mixed completely to obtain a homogeneous phase of polymer, and drug. It should be pointed out that Carbopol gel can be used as advanced drug delivery because of good rheological properties resulting in long statement on the administration site [52]. Other advantages of Carbopol gel as a carrier for the delivery of drugs are compatibility with many active ingredients, bioadhesive properties, good thermal stability, good patient acceptability, [52] excellent organoleptic characteristics, wide concentration interval and characteristic flow behavior. Flow properties of Carbopol allow injection of gel directly into the periodontal pocket using a syringe and mucoadhesive behavior of Carbopol provide prolonged retention within the pocket, and sustained release of therapeutic agent within this environment. Moreover, Carbopol 974P is an optimal vehicle for dental anti-inflammatory hydrogel and products on the base of Carbopol 974P are certified as fit for oral administration and for contact with mucosa. Participants in the control group received similar gel without AZM (1% Carbomer gel 974P). Test and control gels were identical in color and transparency. Before injection of the in situ gels, the sites were isolated from the saliva by applying cotton rolls and then were dried with an air syringe. In both groups, 0.2 ml prepared gels were applied into the periodontal pockets in single-rooted teeth with insulin syringes and blunt-tip cannulas. The gels were injected twice with a time interval of 20 minutes per site. After placement of the in situ gels, subjects were educated to delay brushing their teeth for 8 hours and to eschew from chewing hard or sticky food, and using interdental cleaning device for 7 days. Moreover, periodontal dressings were not utilized after local delivery.

**Statistical Analyses**

Data from a total of 120 participants were available for analyses. All data were analyzed using the Statistical Package for the Social Sciences (SPSS version 21.0, SPSS, Chicago, IL, USA) software. The data were evaluated on the subject basis.

1. For evaluation and comparison of PD and CAL parameters: Results were expressed as mean ± standard deviation. The data were statistically analyzed using two-way analyses of variance (ANOVA). Tukey's test was applied to evaluate inter-groups comparisons. Paired t-test was used to compare the baseline visit with follow-up visits (intra-group comparisons).

2. For evaluation and comparison of MGI and PBI parameters: The Kruskal-Wallis analysis was applied to compare the parameters recorded at different time points. Significance of differences between the four groups at each time-point was analyzed by Mann-Whitney test for each adjunctive medication independently. The significance of differences was sought using the Friedman test for intra-group assessments, in changes between baseline and follow-up visits. In addition, the Wilcoxon test was carried out to assess the changes occurring in different time intervals from day 0 in each group.
3. For evaluation and comparison of *P. gingivalis* and *A. actinomycetemcomitans* counts: Results were expressed as mean ± standard deviation. The data were statistically analyzed using one-way analyses of variance (ANOVA). Tukey's test was used to assess inter-groups comparisons. Paired t-carried out to compare the baseline visit with follow-up visits (intra-group comparisons). Differences with a P value <0.05 at a confidence level of 95% were considered significant.

**Ethical Consideration**

The study protocol was approved by the Committee of Ethical Affairs of the Faculty of Dentistry and Torabinejad Dental Research Centre at the Isfahan University of Medical Sciences, Isfahan, Iran. This clinical trial was conducted according to the principles outlined in the Declaration of Helsinki on experiments involving human subjects. This study has been published on ClinicalTrials.gov with registration number: NCT01921738.

**3. Results**

Figure 1 depicts the participation of individuals during the study. The baseline demographic and clinical characteristics of each group are illustrated in Table 1. One hundred twenty patients in the age group of 20 to 55 years were enrolled in the study and randomly assigned in four groups (1, 2, 3, and 4). Clinical parameters were evaluated at baseline and at 1, 2, 3, and 4 months after SRP. Microbiologic parameters were recorded at baseline in 40 patients (10 samples per group) and at 4 months after SRP (totally 80 samples). All subjects were treated according to the protocol and complied with the follow-up visits. No adverse drug reactions were reported at any time and no patient reported any discomfort.

![The consort E-flowchart](image-url)
Clinical Variables

Mean values at each study visit are shown in Table 2.

PD and CAL

PD and CAL changes were statistically significant in all four groups compared to baseline at all time intervals. Repeated-measures ANOVA demonstrated that time, type of medication, and the interaction of these two factors had significant effect on overall mean PD and CAL changes for all four groups at different time points ($P <0.001$). Intergroup comparison by Tukey test for PD and CAL changes showed significant differences between the groups except difference between the placebo systemic and placebo locally groups (Table 3). There was statistically significant improvement for the observed parameters in all four groups at 4 months; however, the data indicated the most improvement trend in group 1 and group 2, respectively (Figure 2, 3).

Fig. 2. Mean periodontal probing depth in study groups during experimental periods.

Fig. 3. Mean clinical attachment level in study groups during experimental periods.

MGI and PBI

Intragroup assessment showed significant differences at all time intervals compared to baseline in all four groups ($P <0.001$). Additionally, intergroup comparison demonstrated greater reductions in group 1 and group 2, respectively (Figure 4, 5).
Fig. 4. Mean papilla bleeding index in study groups at different time points.

Fig. 5. Mean modified gingival index in study groups at different time points.

**Microbiological Variables**

Out of the 40 patients positive for the two periodontal pathogens *P. gingivalis* and *A. actinomycetemcomitans* (10 patients from each group), 40 samples were available at baseline and 40 samples at the 4-month visit.

**A. Actinomycetemcomitans and P. Gingivalis Counts**

One-way analyses of variance (ANOVA) detected significant differences at the end of 4 months compared to baseline in frequencies of detection of target pathogens between four groups ($P < 0.001$). Moreover, Paired t-test
indicated statistically significant reductions in mean *P. gingivalis* and *A. actinomycetemcomitans* between baseline and 4-month visit in all four groups (Table 4 and Figure 6). Additionally, results of intergroup comparison using Tukey's test are given in Table 5.

![Fig. 6. Mean changes in microbiologic parameters (count of Aa and Pg) between baseline and 4-month visit in study groups.](image)

*Aa = A. actinomycetemcomitans; Pg = P. gingivalis.*

**Safety**

Adverse events were not occur in all the groups during the course of study.

4. Discussion

To the best of our knowledge, this is the first study in which the efficacy of systemic and locally AZM at 1% concentration has been evaluated as an adjunct to SRP on both clinical and microbiologic parameters in CP patients harbouring *A. actinomycetemcomitans* and *P. gingivalis* simultaneously. The data from this trial indicated that the addition of AZM (systemic and locally) to SRP for the treatment of moderate to severe chronic periodontitis resulted in statistically significant improvement in parameters investigated. Although treatment strategies in all four groups seemed to benefit the patients, the clinical and microbiologic results as shown by the intergroup comparison revealed clear differences between test and control groups. AZM suppresses interleukin-12p40 expression in lipopolysaccharide and interferon-y stimulated macrophages. This may explain a mechanism for the regulation of the anti-inflammatory effects of AZM in macrophages [53]. Furthermore, pro-inflammatory cytokines, such as TNF-Q, IL-1B, IL-6 and IL-8, are known to be suppressed by AZM [54-55]. In addition to this, compared with long courses of other antibiotics, the 3-day regimen of AZM increases patient compliance. Moreover, compared to a systemic regimen, local drug delivery provides less undesirable reactions, favorable patient compliance, and high intrasulcular drug concentration with minimum systemic exposure [56-57]. Because of these factors, this study was designed to assess and compare the effects of SRP plus 1% AZM via local drug delivery (group 1) and SRP combined with a 3-day regimen of AZM (group 2) on parameters investigated in patients with CP. AZM possesses host-modulator properties in the treatment of periodontitis and chronic inflammatory diseases. Recent studies showed that placement of in situ gel of 0.5% AZM after SRP compared to SRP alone enhanced the clinical results and improved microbiologic parameters. [31, 47]. In our study, AZM groups resulted in statistically significant improvement in both clinical and microbiologic parameters. The results of this study demonstrated a statistically significant reduction in PBI in the test and placebo groups compared to baseline at all time intervals. At the end of study (4 months), a statistically significant improvement in PBI scores of 3.76 + 0.43, 3.70 + 0.46, 3.36 + 0.61, and 3.43 + 0.72 was observed for groups 1, 2, 3, and 4, respectively, compared to baseline. The differences between the groups reached the level of significance at 2, 3, and 4 months. At 2 months, the difference between group 1 with other groups was statistically significant. At 3 months, the difference between group 1 with other groups and the difference between groups 3 and 4 reached the level of significance. At 4 months, the difference between groups 1 and 2 as
well as groups 3 and 4 failed to reach the level of significance. Although intergroup analyses showed significant differences between group 1 with other groups at 2, 3, and 4 months, no statistically significant difference was observed between group 1 and group 2 at the end of 4 months. Further, the difference between group 2 and the control groups (placebo groups) reached the level of significance at 4 months. In general, reduction in PBI score was higher in groups 1, 2, 4, and 3, respectively. Moreover, the results of the present study show that the difference between the test and control groups was statistically significant at the end of 4 months. These findings are in agreement with Smith et al., who found improvement in PD and bleeding on probing (BOP) after adjunctive administration of systemic AZM. Conversely, Mascarenhas et al.\cite{29} showed that, although systemic AZM combined with SRP in the management of CP in smokers resulted in PD reduction and CAL gain, no statistically significant changes regarding mean BOP was observed. This may be due to the fact that the temporary gingival vasconstriction induced by nicotine, and thereby smokers present with reduced BOP. In addition to this, the authors noted that the improvement in BOP scores was more affected by the efficacy of SRP than the effects of systemic AZM. Further, Han et al.\cite{58} showed that adjunctive systemic AZM in combination with SRP did not significantly improve clinical (PD, CAL, BOP) and microbiologic and biochemical parameters. However, it should be noted that in this study microbiologic assessment was done for 2 teeth and gingival crevicular fluid matrix metalloproteinases-8 (GCF MMP-8) levels were determined just for one tooth. Recently, Venkatesh et al.\cite{35} who evaluated in vivo and in vitro effectiveness of Azithromycin smart gel for the treatment of CP, found improvement in clinical parameters including gingival index (GI), probing pocket depth, clinical attachment level, bleeding index and plaque index. Additionally, according to the authors, the other advantages of application of AZM gel may be appreciated by lowest rate of side effects, decreases the usual surgical procedures, in turn, promotes patient compliance. This study demonstrated a statistically significant reduction in MGI in the test and control groups compared to baseline at all time intervals. At the end of study (4 months), a statistically significant improvement in MGI scores of 3.93 + 0.25, 3.76 + 0.43, 3.40 + 0.56, and 3.40 + 0.62 was observed for groups 1, 2, 3, and 4, respectively, compared to baseline. The differences between the groups reached the level of significance at 2, 3, and 4 months. Intergroup comparison presented significant differences between group 1 with other groups at 2, 3, and 4 months; however, no statistically significant difference was observed between group 1 and group 2 at the end of 4 months. It seems that groups 1 and 2 affected on MGI in a similar fashion after 4 months. It appears that the effectiveness of systemic and local AZM became stabilized 3 months after administration. Moreover, the difference between group 2 and the control groups (groups 3 and 4) reached the level of significance at 4-month. Nonetheless, there were no significant differences between groups 3 and 4 at any time point. Consequently, reduction in MGI score was higher in group 1 compared with three other groups. At the end of our study (4 months), a statistically significant difference was observed between the test (groups 1 and 2) and placebo groups (groups 3 and 4). It is reasonable to assume that locally applied of 1% AZM showed more significant anti-inflammatory action in comparison with systemic AZM. The improvement in MGI scores at the end of our study was much better than the improvement in MGI scores reported by Pradeep et al.\cite{28} they mentioned that subgingivally delivered 0.5% AZM on the base of PLGA gel, probably because of the acidic degradation of polylactide and polyglycolide may contribute to the sustained inflammation in the test group, which resulted in less improvement in MGI scores in the test group in comparison with the control group. On the other hand, Dastoor et al.\cite{29}, announced that systemic administration of AZM during surgical therapy in heavy smokers yielded significantly better wound healing indices (WHI) at 1 month, significantly less GI at 2 week, and sustained reductions of red-complex bacteria with trypsin-like enzyme activity at 3 months in the test group compared to the control group. The authors explained that this may be due to the fact that smokers tend to harbor more periopathogenic bacteria and more plaque than nonsmokers. Additionally, none of the patients decreased their smoking habit which, in turn, overwhelmed the initial short-term benefit of the antibiotic, resulted in recolonization of pockets by pathogenic bacteria. As a result, smoking promotes the disease process to continue. It can be hypothesized that the AZM’s effect leads to a rapid reduction in the inflammatory lesion. It is known from literature that AZM play a substantial role in immune-modulating and resolution of inflammation\cite{42}. A significant reduction in PD and gain in CAL were observed within four groups compared to baseline at all time intervals. In the present study, the reduction in PD and gain in CAL were 5.14 + 0.46 mm, 4.58 + 0.62 mm, 2.28 = 0.63 mm and 2.73 + 0.89 mm for groups 1, 2, 3, and 4, respectively, at the end of 4 months. However, the reduction in PD and CAL gain were statistically significant in the test and placebo groups at each study period (P <0.05); more pronounced PD reduction and CAL gain were observed in the test groups (groups 1 and 2, respectively). These findings may be explained by the sustained effect of AZM\cite{27,37}. Further, the results of intergroup comparisons revealed a statistically significant PD reduction and CAL gain in groups 1, 2, 4, and 3, respectively. Moreover, this study demonstrated no statistically significant differences for decrease in PD and gain in CAL between placebo groups (groups 3 and 4) at any time point (P >0.05). these results are in agreement with previous studies that demonstrated that subgingivally delivered 0.5% AZM combined with SRP provided significant improvement in clinical outcome (i.e., PD reduction and CAL gain) in the treatment of chronic periodontitis among smokers.\cite{26,49}, and non-smokers.\cite{24,28,35,44} With regard to microbiologic parameters, the decrease in A. actinomycetemcomitans and P. gingivalis was statistically significant in all groups compared to
baseline at 4 months after completion of SRP. One possible explanation for these findings can be that, throughout the study period, all patients received adequate periodontal maintenance therapy, including SRP and strict oral hygiene instruction. In addition to this, it has been suggested that red-complex bacteria were significantly reduced by SRP. Nonetheless, the most marked decrease in counts of A. actinomycetemcomitans and P. gingivalis was observed in the adjunctive use of 1% locally AZM. This is in accord with data in the literature suggesting that local drug delivery can enhance eradication or significant reductions of subgingival pathogens by 100-fold accumulation in subgingival space in comparison with systemic therapy [61,62]. Our results are in accordance with Oteo et al. [44] who found clinical improvement in parallel to microbiological benefits after the adjunctive use of systemic AZM with SRP. The value of the adjunctive use of AZM after completion of SRP might be appreciated in terms of facilitated longterm maintenance of shallow PD and CAL gain which, in turn, yields decreased counts of putative periodontal pathogens like A. actinomycetemcomitans and P. gingivalis. In general, our results have shown convincing superiority of adjunctive use of AZM with mechanical debridement over conventional treatments.

5. Conclusion

The present study indicated that SRP plus the adjunctive use of azithromycin (either locally applied or systemic) enhanced clinical outcomes, e.g., PD reduction, CAL gain, reduction in PBI and MGI, and improved microbiologic parameters, as shown by the decrease in tissue-invasive pathogens, especially Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis compared with SRP plus placebo in patients with moderate-severe CP. On the other hand, better clinical and microbiological outcomes were obtained with subgingivally delivered 1% azithromycin than systemic AZM. Hence, we can conclude that within the limitations of our study, patients with untreated moderate-severe CP harbouring A. actinomycetemcomitans and P. gingivalis in their subgingival biofilm may benefit from the locally applied and systemic administration of azithromycin in conjunction with SRP. Because the follow-up for the present study was only 4 months, it would be interesting to evaluate how long the initial clinical and microbiological benefits could be sustained in each group. More expanded studies, using different vehicles and concentration are recommended to determine the clinical and microbiological effects of locally applied AZM in patients with CP and smokers. An attempt should be made to formulate smart gel of azithromycin in the treatment of CP. Finally, it is important to elucidate the role of AZM in the management of peri-implantitis.

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