Journal of Dental Sciences (2021) **16**, 691–699

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

Original Article

Evaluation of the efficacy of lycopene gel compared with minocycline hydrochloride microspheres as an adjunct to nonsurgical periodontal treatment: A randomised clinical trial

Aya Ali [a], Saif Saliem [b], Ali Abdulkareem [b*], Hani Radhi [a], Sarhang Gul [c]

[a] *College of Dentistry, Mustansiriya University, Baghdad, Iraq*
[b] *College of Dentistry, University of Baghdad, Baghdad, Iraq*
[c] *College of Dentistry, University of Sulaimani, Sulaymaniyah, Iraq*

Received 15 August 2020; Final revision received 15 September 2020
Available online 30 September 2020

KEYWORDS
Antioxidants;
Lycopene;
Minocycline hydrochloride;
Nonsurgical periodontal debridement;
Clinical trial

Abstract *Background/purpose:* The prescription of antibiotics as an adjunct to mechanical periodontal therapy in patients with severe periodontitis is recommended; however, the side effects of antibiotics are a major concern. The aim of this study was to evaluate the efficacy of lycopene (Lyc) antioxidant gel versus minocycline hydrochloride microspheres (ARISTIN) as an adjunct to the nonsurgical treatment of periodontitis.
Materials and methods: Three identical periodontal pockets/patient received root surface debridement followed by the random application of either ARISTIN, Lyc, or placebo gel (control, Ctrl). Clinical parameters, plaque index, bleeding on probing, probing pocket depth, and clinical attachment loss, were recorded at the baseline and after 30 days. Additionally, the levels of interleukin-8 (IL-8), matrix metallopeptidase 9, and tissue inhibitor of metalloproteinases 1 (TIMP1) in gingival crevicular fluid samples were assessed at the same time points.
Results: Twenty-three patients with periodontitis completed the study. Both ARISTIN and Lyc treatments showed significantly greater gains in attachment (1.94 ± 1.33 and 1.72 ± 0.88, respectively) than the Ctrl treatment (1.04 ± 0.96). Compared with those in the Ctrl, only ARISTIN showed a significant reduction in IL-8 level, whereas TIMP1 levels were significantly upregulated in the Lyc gel and ARISTIN sites. The effect size estimation indicated that Lyc gel exhibited considerably greater efficacy than the Ctrl gel.

* Corresponding author. College of Dentistry, University of Baghdad, Bab Al Moatham, Medical City of Baghdad, P.O. Box 1417, Baghdad, 10011, Iraq. Fax: +964 14140738.
E-mail address: ali.abbas@codental.uobaghdad.edu.iq (A. Abdulkareem).

https://doi.org/10.1016/j.jds.2020.09.009
1991-7902/© 2020 Association for Dental Sciences of the Republic of China. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Conclusion: Lyc gel and ARISTIN offer almost equal improvement in both clinical and biochemical parameters of periodontitis.
© 2020 Association for Dental Sciences of the Republic of China. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

The hallmark of periodontitis is the formation of periodontal pockets as collateral damage, which is mainly caused by an aberrant immune response to the dental biofilm.[1] The presence of pathogenic bacteria and their virulence factors, such as lipopolysaccharide, upregulates the release of inflammatory cytokines, including interleukin (IL)-8.[2] The persistent release of these cytokines, with increased oxidative stress (OS), potentially compromises the integrity of the epithelial-barrier lining the pocket.[3] The loss of the epithelial-phenotype is attributed to an epithelial—mesenchymal transition (EMT) that is characterised by the downregulation of epithelial markers and acquisition of the mesenchymal-phenotype.[4] The involvement of the EMT in the pathogenesis of periodontitis has been suggested by *in vivo* and *in vitro* studies.[5,6] This process can be reversed by eliminating the triggering factors and modulated by certain drugs, including antioxidants.[7]

In a healthy individual, tissue remodelling by matrix metalloproteinases (MMP) is controlled by tissue inhibitor of metalloproteinases (TIMP) but, with an increased bacterial load in the periodontal pocket, this balance is disrupted, which consequently increases the susceptibility to periodontal tissue destruction.[8] MMP9 is associated with periodontitis[9] and the level of MMP9 increases with the severity of the disease.[10]

The basic principle of nonsurgical periodontal treatment is the removal of bacterial load, which allows the body to return to the healthy state.[11] However, the effectiveness of this approach is not always guaranteed owing to several reasons, such as operator dexterity and the presence of certain bacteria, such as *Aggregatibacter actinomycetemcomitans*, that can re-colonise the root surface rapidly after treatment.[12,13] Furthermore, some of these bacteria invade periodontal tissues, in which case they are difficult to remove by mechanical therapy.[14] Therefore, several therapies have been proposed as adjuncts to scaling and root planing (SRP). Since the late 1970s, the interest in using locally delivered antimicrobials to treat periodontal pockets has increased.[15] A recent systematic review and meta-analysis on the use of antimicrobials in different formulations as adjunctive agents to SRP showed that these agents enhance clinical outcomes with minimal side effects.[16] Among these agents, minocycline hydrochloride microspheres (MM) have been reported to be effective in reducing probing pocket depth (PPD) and increasing attachment gain.[17,18] The axial role of certain bacterial commensals in balancing a healthy subgingival flora has been reported,[19] which can be changed non-specifically by antimicrobials.

The use of antioxidants as adjuncts to periodontal treatment has been proposed recently as an alternative to antimicrobials.[20] Lycopene (Lyc) is an antioxidant extracted from tomato that has a potential therapeutic effect for the treatment of periodontitis when applied locally[21,22] or systemically.[20,23] To the best of our knowledge, studies on the use of antioxidants as adjuncts to conventional periodontal therapy are limited. The rationale of this study is based on the potential use of Lyc gel as an adjunct to SRP in the treatment of periodontitis. The aim of this pilot trial was to evaluate the efficacy of Lyc gel as an adjunct to SRP based on clinical and biochemical markers of periodontitis. This was investigated by measuring the effects of Lyc on clinical parameters and selected inflammatory cytokines in gingival crevicular fluid (GCF) compared with the controls (SRP [control, Ctrl] and SRP + MM [ARISTIN]).

Materials and methods

Study design and population

A double-blind, split-mouth, randomised clinical trial with three arms was conducted at the Department of Periodontics, College of Dentistry, University of Baghdad from March 2019 to January 2020. Ethical approval was obtained from the ethics committee (Ref #013618, 08/01/2019) of the College of Dentistry, University of Baghdad in accordance with the Declaration of Helsinki and CONSORT 2010 checklist. The clinical registration number is #NCT03964935 at www.clinicaltrials.gov/. The aims, design, and methods of the current study were explained to the potential candidates who were invited to participate and, when agreement was obtained, they were asked to sign a consent form before commencing any treatment.

Participants were recruited from patients referred for periodontal treatment and eligible individuals were selected according to the inclusion/exclusion criteria. The included participants were systemically healthy, non-smokers, and not pregnant or lactating, had not taken any drugs in the past 3 months, had no previous or unsuccessful periodontal treatment leaving a residual PPD >4 mm with bleeding, and were able to consent (Fig. 1). Additionally, only single-rooted teeth with infrabony pockets at interproximal sites were included. For pockets between adjoining surfaces, only one of these was selected to avoid possible overlap of the locally delivered agents.

According to the definition of periodontitis adopted by the American Academy of Periodontology and European Federation of Periodontology,[24] the participants exhibited the following diagnostic features based on the clinical criteria and periapical radiography:

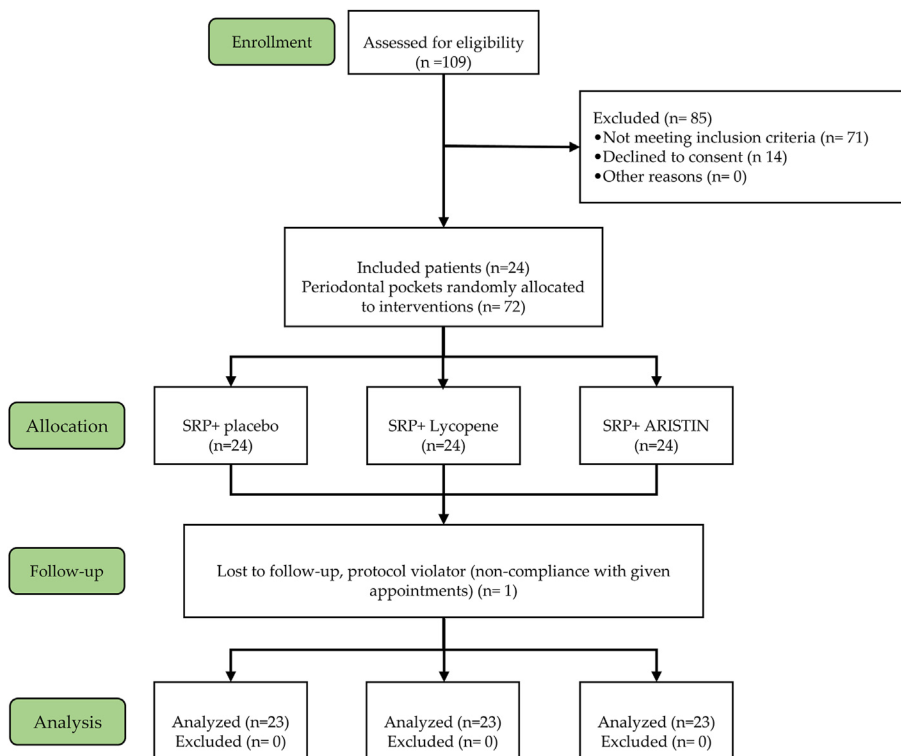


Figure 1 Flow diagram of the study.

- Generalised periodontitis (>30% involvement)
- Stage II—III (bone loss extending to the coronal or middle third of the root)
- Grade B (moderate rate of progression)
- Currently unstable (PPD $\geq$ 4 mm with bleeding on probing [BOP]), any pocket without bleeding was excluded
- No specific risk factors (smoking and diabetes mellitus)

Randomisation of the selected sites was performed using the lottery method (by A.A.A.). The number of teeth to be included in the treatment was written on pieces of paper, which were folded and placed in an opaque bag, and the same was done for the treatment modalities. A paper from each bag was drawn out to determine the tooth and corresponding therapy. Each site that received one of the treatment modalities was assigned a letter, 'A' for Ctrl, 'B' for ARISTIN, and 'C' for Lyc. Subsequently, all records for each site were sorted under the assigned letter to ensure the blindness of the statistician.

Clinical parameters and examiner calibration

Multiple clinical parameters were used to investigate the primary outcomes, including plaque index (PI),[25] BOP,[26] PPD, and clinical attachment loss (CAL). Clinical parameters were recorded following the Quadrant Tooth Site system; six sites per tooth were examined. CAL was determined by measuring the distance from the lower border of a pre-fabricated stent to the base of the pocket.

On the first visit, pre-treatment, initial clinical parameters were recorded. First, PI was recorded, followed by PPD and BOP, to reach a diagnosis, which was confirmed by periapical radiography for the suspected sites. All clinical parameters were recorded using William's periodontal probe (MEDESY, Maniago, Italy). Impressions of the teeth were taken to fabricate a stent to measure CAL. This was followed by providing the patient with oral hygiene instructions, which included advice regarding toothbrushing and the use of interdental aids, while the use of mouthwash was prohibited during the study period. To ensure the blindness of the data collector, all treatments were performed by another clinician (A.H.A.). The treatment started with supragingival scaling using an ultrasonic scaler, DTE® D2 LED, (Guilin Woodpecker Medical Instruments Co Ltd, Guilin, China), followed by coronal polishing. Each patient was instructed to return after 7 days to complete the planned treatment.

The next visit was the baseline for the study. First, compliance with the given oral hygiene instructions was evaluated using PI. For non-compliant patients, instructions were reinforced and the treatment visit was postponed for another week. For compliant participants, baseline data at selected sites (PI, PPD, BOP, and CAL) were recorded. This was followed by collecting the GCF samples. Each of the selected sites underwent thorough root surface debridement using a Gracey curette (MEDESY, Maniago, Italy), which was followed by the application of either placebo gel (Ctrl) or Lyc gel (100 μL), both of which were delivered by an insulin syringe with a blunt needle, or ARISTIN; the patients were blinded to the treatment. After 30 days, clinical parameters and GCF samples were collected again and coded data were sent to the statistician (H.S.R.) who blindly analysed the results.

Prior to the trial, one of the authors (S.S.J.) and one of the clinicians (A.H.A.) participated in calibration sessions for clinical parameters (PI, BOP, PPD, and CAL). The examiners met prior to these calibration sessions to discuss the criteria and methods of measurement. Calibration sessions were conducted using five volunteers not included in the study. Probing pressure potentially increases the PPD;[27] thus, a minimum of 15 min was allowed between repeated measurements by the two examiners to minimise any possibility of error.[28] Alignment for BOP was conducted in a separate session. The weighted kappa (κ) was considered acceptable when it was $\geq$0.70 for all indices. In the case of disagreement, the methods were discussed and the calibration session was repeated on another date.

GCF collection and enzyme-linked immunosorbent assay (ELISA)

The site of the tooth was isolated with a cotton roll and debris and thin plaque film were removed manually with a gentle wiping movement using clean gauze.[29] The site was then dried and the GCF was collected using the intracrevicular method. Tweezers were used to gently insert paper strips, PerioCol (Oraflow Inc., Hewlett, NY, USA), into the depth of the pocket until minimal resistance was felt; then, they were left in the site for 30 s, after which the PerioCol was removed from the pocket and inspected for any sign of contamination with saliva or blood. Contaminated papers were discarded and the procedure was repeated after 30 min; otherwise, the samples were transferred to a pre-weighed Eppendorf tube that contained 200 μL of phosphate-buffered saline (PBS) (Euro Clone, Milan, Italy) and stored at −20 °C. The volume of the collected GCF was determined as previously described,[30,31] which is essential for the proper calculation of the concentration of each marker. Briefly, the weight of the collected GCF was calculated according to the following formula:

$$W_{GCF} = W_2 - W_1,$$

where W_{GCF} is the weight of GCF (μg), W_2 is the Weight of Eppendorf tube containing 200 μL of PBS + PerioCol after collecting GCF, and W_1 is the pre-weighed Eppendorf tube containing 200 μL of PBS. Then, the weight of GCF alone was calculated by subtracting the weight of the PerioCol paper from W_{GCF}. Finally, the volume of GCF was converted from μg to μL, assuming that the density of GCF is (1 mg/mL). This was performed using the formula 'Volume = Mass/Density'.

The samples were thawed and then centrifuged at 400–500 *g* for 4–5 min and concentrations of IL-8, MMP9, and TIMP1 were determined using ELISA kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. The optical density (OD) was determined using a microplate reader (GloMax®, Promega, Madison, WI, USA) at 450 nm, which was converted into a corresponding concentration (ng/mL) using a specific equation for each cytokine obtained from plotting a standard curve. This value represented the concentration (in 1 mL) of the mediator eluted in 200 μL; therefore, the concentration of the cytokine in the original GCF samples was calculated according to the previously measured GCF volume as follows:

ELISA output $\times$ 0.2/GCF volume (μL).[32]

Study interventions and outcomes

Three interventions were used: First, ARISTIN (MM), 1 mg (oraPHARMA, Bridgewater, NJ, USA). Second, Lyc gel, the study drug, prepared according to a previously reported method without any modifications.[22] This procedure was used to convert pure Lyc powder (IBIS CHEMIE International, Mumbai, India) into gel. The concentration of Lyc in the final product was 2% at neutral pH (7.4). Third, the same steps were repeated to prepare a non-active control (placebo) gel without adding the active ingredient, i.e., Lyc powder.

The clinical parameters were set as the primary outcomes of the current study. The outcomes were measured by calculating the differences in these parameters between the baseline and endpoint of the study (after 30 days). In brief, gain of attachment (ΔCAL), reduction in PPD (ΔPPD), and reduction in the percentage of BOP (Δ%BOP) were used to investigate the effect of each treatment modality. Concentrations of inflammatory cytokines in GCF samples were considered as secondary outcomes and were calculated similarly to the primary outcomes.

Sample size

Calculation of the required sample was based on the changes in CAL measurements per site. A preliminary study was initially conducted on six patients to determine the mean and standard deviation of CAL for the Lyc and Ctrl groups at the baseline and endpoint. The CAL value of the Ctrl group was 4.325 ± 1.166, whereas that of the Lyc group was 3.34 ± 1.079. The calculated sample size was 22 sites per group at power 0.8 and 0.05 α-error probability. The total number of required sites to reject the null hypothesis was 66 but 24 sites were included per group to avoid dropdown in the sample. The sample size calculation was performed using G*Power (version 3.1.9.2).

Statistical analysis

Descriptive statistics were used to analyse the data in terms of mean, standard deviation, frequency, and percentages. Before inferential analysis, a normality test showed that the data were not normally distributed; thus, nonparametric statistics were selected for analyses. For continuous variables, intragroup differences were compared using the Wilcoxon signed-rank test, whereas intergroup differences were compared using the Kruskal–Wallis test followed by the Dunn-Bonferroni post hoc test. Changes in PI scores over multiple visits within each group were determined using the Friedman and Dunn-Bonferroni post hoc tests. Fisher's exact test was used to analyse the dichotomous variable (BOP). The effect size of the difference in means was calculated using Cohen's *d* and the Koopman asymptotic score was used to calculate the effect size of changes in BOP%. Differences were considered significant when the

P value was less than 5%. All statistical analyses were performed using GraphPad Prism (version 8.4.3) software.

Results

Recruitment of patients and demographic characteristics of the study groups

A total of 109 patients were assessed for eligibility and 24 patients were recruited after excluding 85 patients (Fig. 1). The final analysis of the study included 23 patients and 69 sites, which were equally distributed among the three groups. One patient did not comply with appointments and was thus excluded from the final analysis (Fig. 1). The demographic variables of the participants are summarised in Table 1. No adverse events were reported as a consequence of this study.

Intragroup comparisons of clinical and biochemical parameters

PI scores were significantly lower ($P < 0.05$) at the baseline visit when treatment was provided. All groups showed an increase in PI at the end of the study; however, these increases were not significant compared with baseline scores (Fig. 2A). All treatments resulted in a significant reduction ($P < 0.05$) in the percentage of BOP at the end of the trial compared with their baseline score. For CAL and PPD, all groups showed significant differences ($P < 0.05$) at the endpoint compared with their baseline scores (Table 2). The concentrations of cytokines in GCF at the baseline and endpoint of the trial showed that patients in the Ctrl group only exhibited a significant difference in the level of MMP9. For combined SRP with ARISTIN or Lyc, both adjunctive drugs showed significantly reduced ($P < 0.05$) levels of IL-8 and MMP9 and increased levels of TIMP1 at the endpoint compared with the baseline (Table 2).

Intergroup comparisons of clinical and biochemical parameters

At the baseline, there were no significant differences in two clinical parameters, PPD and CAL, among all groups (Fig. 2B). ARISTIN and Lyc showed a significantly greater gain in attachment ($\Delta CAL = CAL_{baseline} - CAL_{endpoint}$) than the Ctrl. Additionally, both drugs had a high effect size (0.774–0.792) compared with the Ctrl (Table 3). Although no significant differences in ΔPPD were apparent when the Ctrl was compared with ARISTIN and Lyc, a moderate effect size was observed between ARISTIN (0.655) or Lyc (0.461) and the Ctrl (Table 3). The same pattern was observed in association with Δ%BOP, in which ARISTIN and Lyc were more effective than the Ctrl (Table 3).

Regarding changes in the concentration of cytokines, ARISTIN and Lyc failed to cause significant changes in the level of MMP9 compared with the Ctrl; however, they showed a moderate effect size (0.532–0.482) compared with the Ctrl. For ΔIL-8, only ARISTIN resulted in significant changes ($P = 0.016$) in the level of this cytokine at the endpoint, with a high effect size (0.971) compared with the Ctrl (Table 3). A significant alteration in the level of TIMP1, with a high effect size, was observed between ARISTIN ($P = 0.041$, effect size = 0.786) or Lyc ($P = 0.038$, effect size = 0.897) and the Ctrl (Table 3).

Table 1 Demographic data of the patients.

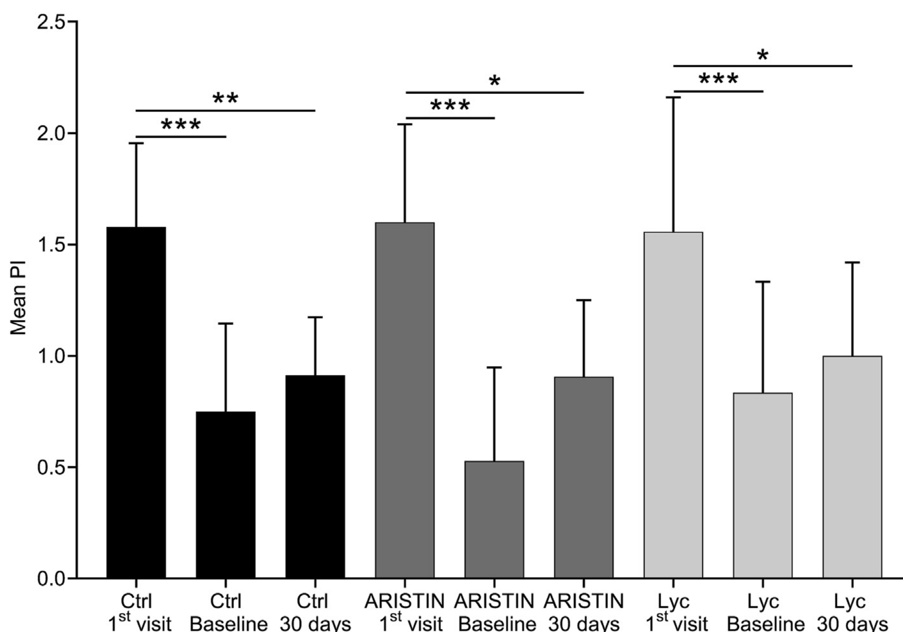
Sex	N	%		
Male	15	66		
Female	8	34		
Age groups (years)			Mean ± SD	Range
30-39	7	30.4	33.1 ± 2.4	33–36
40-49	6	26.1	42.5 ± 1.8	40–45
50-59	7	30.4	52.3 ± 2.3	50–55
>60	3	13.0	66.3 ± 5.1	62–72
Total	23	100	45.7 ± 11.5	30–72

N, Number.

Discussion

The main results of this phase I clinical trial showed that Lyc gel and ARISTIN were equally effective in improving clinical CAL measurements and levels of biomarkers involved in periodontal tissue destruction compared with the Ctrl group. The exact etiopathological mechanism of periodontitis has not been fully elucidated; however, it is well-recognised that the destruction of periodontal tissues is a collateral event to an intense immune response to pathogenic bacteria or their virulence factors.[33] Tissue integrity is maintained by homoeostasis, which involves an antioxidant system that counteracts the adverse effects of reactive oxygen species. Increased OS has been proposed to be associated with periodontal diseases.[3] Data regarding the effectiveness of locally delivered antioxidant agents as an adjunct to nonsurgical periodontal treatment are limited. The aim of this study was to test the hypothesis that the local application of Lyc as an antioxidant in periodontal pockets potentially improves periodontal status. The selection of Lyc was based on reported results for using this drug as supportive therapy for SRP, both systemically[34] and locally.[21,22]

Despite satisfactory results using subgingival antimicrobials to treat periodontal diseases, an *in vitro* study has shown that subgingival bacteria in steady-state biofilms require a 250-fold greater concentration of antimicrobials to inhibit their growth than bacteria outside biofilm, and approximately half of these bacteria remain viable.[35] MM is an antimicrobial that has been shown to be effective as an adjunct therapy to SRP in many studies;[17,18,36] therefore, it was selected as a positive control for this study. The main mechanism of action of this antimicrobial is the elimination of Gram-negative anaerobes.[37] A reduction in the bacterial load in the subgingival domain is of paramount importance in periodontal therapy as this provides the immune system enough time to overcome the remaining bacteria and decrease the level of the cytokines, thereby restoring the healthy state.[11] It is important to acknowledge that, in clinical trials, besides the *P* value, the effect size needs to be taken into consideration, especially when the sample



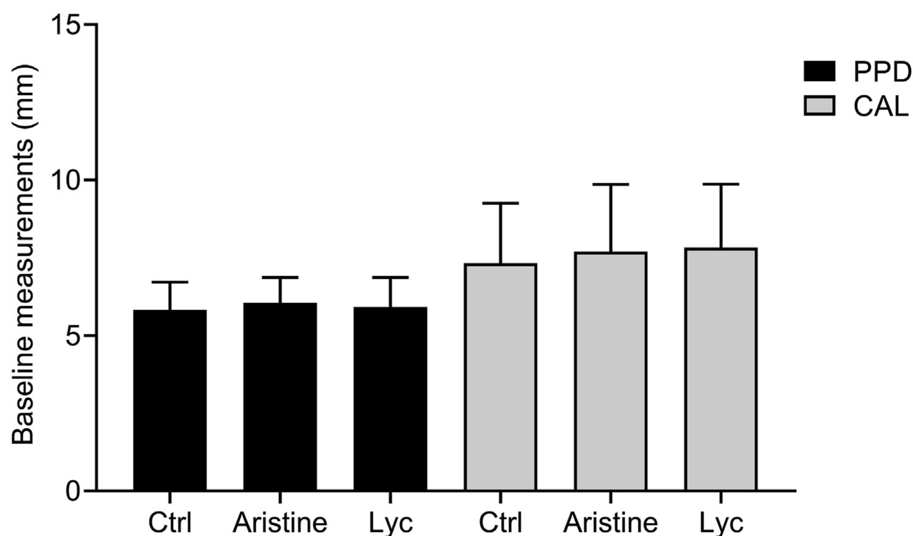


Figure 2 (A) Plaque index scores significantly decreased from the first to baseline visit, within 7 days, for all groups. At the endpoint, after 30 days, plaque index scores for all treatment modalities increased again; however, these scores remained significantly lower than at first visit and non-significantly different from baseline scores (* = $p < 0.05$, ** = $p < 0.002$, *** = $p < 0.001$). (B) Comparison of PPD and CAL measurements for the three groups indicated no significant differences among them at the baseline.

size is small.[38] Therefore, the effect size has been reported in the current study.

The results of this study showed that MM significantly reduced PPD by the end of the trial but there was no significant difference compared with Ctrl and Lyc. This is in agreement with a previous study that indicated no additional benefit of using MM as an adjunct to nonsurgical treatment over SRP alone.[39] However, this disagrees with results from other studies that have shown a significant reduction in PPD in the MM group compared with the SRP group,[17,18,36] which is an inconsistency that could be owing to differences in the sample size and follow-up period. Furthermore, with Lyc, the gain of clinical attachment at the endpoint was almost half the amount of the reduction

Table 2 Intragroup comparison of clinical parameters and cytokines concentrations at baseline and endpoint of the study.

Parameters	Mean ± SD		
Clinical	Ctrl	ARISTIN	Lyc
BOP $_{baseline}$ (N, %)	23, 100	23, 100	23, 100
BOP $_{endpoint}$ (N, %)	13, 57	6, 26	7, 30
p value[a]	<0.001	<0.001	<0.001
CAL $_{baseline}$ (mm)	7.33 ± 1.93	7.69 ± 2.16	7.83 ± 2.04
CAL $_{endpoint}$ (mm)	6.28 ± 1.82	5.76 ± 2.21	6.11 ± 1.98
p value[b]	<0.001	<0.001	<0.001
PPD $_{baseline}$ (mm)	5.82 ± 0.88	6.04 ± 0.82	5.91 ± 0.95
PPD $_{endpoint}$ (mm)	4.00 ± 1.41	3.44 ± 1.12	3.57 ± 1.08
p value[b]	<0.001	<0.001	<0.001
Biochemical[c]			
IL-8 $_{Baseline}$	46.75 ± 28.75	56.87 ± 23.94	59.30 ± 22.50
IL-8 $_{endpoint}$	41.63 ± 24.47	31.97 ± 10.97	43.84 ± 23.73
p value[b]	NS	0.002	0.005
MMP-9 $_{Baseline}$	85.76 ± 23.98	79.79 ± 29.86	82.88 ± 32.98
MMP-9 $_{endpoint}$	70.14 ± 35.95	51.58 ± 26.20	52.13 ± 35.95
p value[b]	0.025	0.001	0.001
TIMP-1 $_{Baseline}$	3.31 ± 1.87	3.31 ± 1.37	3.18 ± 1.29
TIMP-1 $_{endpoint}$	3.86 ± 1.84	5.19 ± 1.59	5.31 ± 1.94
p value[b]	NS	0.004	<0.001

N, number; BOP, bleeding on probing; CAL, clinical attachment loss; PPD, probing pocket depth; IL, interleukin; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; Lyc, Lycopene.

[a] Significance at $p < 0.05$ by Fisher's exact test at 95% confidence interval.

[b] Significance at $p < 0.05$ by Wilcoxon signed rank test.

[c] Concentrations of cytokine (ng/ml).

in PPD, which is similar to previous results from a systematic review of the effectiveness of SRP.[40] Combined Lyc and SRP showed a significant gain in attachment compared with the placebo group. These results are consistent with other trials that also utilised Lyc as an adjunct to the nonsurgical treatment of periodontitis.[21,22] Additionally, both MM and Lyc were more effective in the reduction of BOP compared with SRP alone, but not significantly so.

Microulceration of the epithelial pocket lining exposes the underlying highly vascularised connective tissue, which increases the tendency for bleeding.[1] Additionally, discontinuity in the epithelial layer allows bacteria to enter deeper tissues and cause more damage to tooth-supporting structures.[1] The presence of Gram-negative bacteria, OS, and high levels of inflammatory cytokines negatively affect the epithelial-barrier function of the pocket lining. Exposure of epithelial cells to gingipains, virulence factors of *Porphyromonas gingivalis*, results in the cleavage of epithelial adhesion-molecules[41] and increases the expression of MMP,[42] which enhances the dispersion of the epithelial layer. Furthermore, results from a previous study show the significant downregulation in the expression of major epithelial markers, E-cadherin and β-catenin, associated with the acquisition of the fibroblast-like phenotype following exposure of epithelial cultures to OS.[43] The authors suggested that OS could act alone or synergistically with other inflammatory cytokines to alter the epithelial-phenotype.[43] The mechanism by which Lyc minimised bleeding could be attributed to decreased OS in the pocket microenvironment, which has been previously demonstrated by another study that showed that 8-oxo-2′-deoxyguanosine is restored to almost normal levels after 1 week following the local application of Lyc in periodontal pockets.[22]

The reduction of OS has a positive impact on the tissue that enhances healing and restores the architecture of the tissue.[44] Tissue plasticity is an elusive process that cannot be tracked clinically in humans owing to several technical and ethical issues. However, the events of this process can be predicted indirectly by measuring the level of inflammatory cytokines, which are considered the main driver for the initiation and sustainability of the EMT.[4] Therefore, inflammatory cytokines that are differentially expressed during periodontitis and the EMT[45] were used in this study. Lyc treatment resulted in the significant downregulation of IL-8 and MMP9 levels and significant upregulation of TIMP1 levels, which are known to balance the destructive effect of collagenases;[8] this could provide additional support for the healing effect of Lyc. The change in the level of these cytokines was associated with a significant gain of attachment and reduced bleeding in sites treated with Lyc. Previous studies have demonstrated that SRP significantly upregulates the level of TIMP1 in GCF samples from patients with periodontal disease,[46] which is accompanied by a significant gain of attachment and reduction in PPD.[47] Similar results were obtained in the current study in association with SRP and further upregulation of TIMP1 was observed when SRP was combined with Lyc.

This study lacked the measurement of OS in GCF samples, which could better reflect the response of the periodontal tissue to the treatments. A comparison of alterations in the subgingival microflora before and after each treatment modality requires consideration in future investigations. Additionally, this study only monitored the effect of a single application of Lyc over a short period of time. Thus, studies over a longer period and utilising a larger population are recommended.

Despite the limitations of this study, combining SRP with Lyc gel in the treatment of mild to moderate periodontitis showed promising results. This was reflected by significant differences in clinical and biochemical parameters compared with the controls. Additionally, Lyc was almost as effective as MM in improving outcomes of nonsurgical periodontal therapy. These results suggest that Lyc significantly modulated the expression of inflammatory cytokines in GCF, which positively influenced clinical outcomes. Although antioxidants represent an alternative to antimicrobials in the treatment of periodontitis, the current results should be interpreted with caution, and further

Table 3 Intergroup comparison of the changes in clinical parameters (primary outcomes) and inflammatory mediators' concentration (secondary outcomes) at baseline and at the endpoint of the trial.

Δ Primary outcomes	Non-bleeding sites at endpoint			Comparisons	p value[a]	Effect size[c]
	Ctrl	ARISTIN	Lyc			
BOP (N, %)				Ctrl vs ARISTIN	NS	1.212
	10 (44)	17 (74)	16 (70)	Ctrl vs Lyc		1.200
				ARISTIN vs Lyc		1.026
	Mean difference± SD				p value[b]	Effect size[d]
	Ctrl	ARISTIN	Lyc			
CAL (mm, gain)				Ctrl vs ARISTIN	0.029	0.792
	1.04 ± 0.96	1.94 ± 1.33	1.72 ± 0.88	Ctrl vs Lyc	0.036	0.774
				ARISTIN vs Lyc	NS	0.150
PPD (mm, reduction)				Ctrl vs ARISTIN	NS	0.655
	1.83 ± 1.27	2.61 ± 1.12	2.35 ± 0.98	Ctrl vs Lyc	NS	0.461
				ARISTIN vs Lyc	NS	0.118
Δ Secondary outcomes						
IL-8 ↓				Ctrl vs ARISTIN	0.016	0.971
	5.119 ± 14.14	24.90 ± 25.10	15.46 ± 16.63	Ctrl vs Lyc	NS	0.667
				ARISTIN vs Lyc	NS	0.376
MMP9 ↓				Ctrl vs ARISTIN	NS	0.482
	15.62 ± 24.10	28.21 ± 27.96	30.75 ± 32.11	Ctrl vs Lyc	NS	0.532
				ARISTIN vs Lyc	NS	0.084
TIMP1 ↑				Ctrl vs ARISTIN	0.041	0.796
	0.55 ± 0.92	1.83 ± 2.08	2.13 ± 1.61	Ctrl vs Lyc	0.038	0.897
				ARISTIN vs Lyc	NS	0.135

N, number; BOP, bleeding on probing; CAL, clinical attachment loss; PPD, probing pocket depth; IL, interleukin; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; Lyc, Lycopene.
[a] Significance at $p < 0.05$ by Fisher's exact test at 95% confidence interval.
[b] Significance at $p < 0.05$ by Kruskal–Wallis test.
[c] Effect size by Koopman asymptotic score at 95% confidence interval.
[d] Effect size by Cohen's *d*.

studies are needed to better clarify the feasibility of the antioxidant.

Declaration of Competing Interest

The authors have no funding and no conflicts of interest relevant to this article.

References

1. Ji S, Choi YS, Choi Y. Bacterial invasion and persistence: critical events in the pathogenesis of periodontitis? *J Periodontal Res* 2015;50:570—85.
2. Song B, Zhang YL, Chen LJ, et al. The role of Toll-like receptors in periodontitis. *Oral Dis* 2017;23:168—80.
3. Wang Y, Andrukhov O, Rausch-Fan X. Oxidative stress and antioxidant system in periodontitis. *Front Physiol* 2017;8:910.
4. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009;119:1420—8.
5. Abdulkareem AA, Shelton RM, Landini G, Cooper PR, Milward MR. Potential role of periodontal pathogens in compromising epithelial barrier function by inducing epithelial-mesenchymal transition. *J Periodontal Res* 2018;53:565—74.
6. Nagarakanti S, Ramya S, Babu P, Arun KV, Sudarsan S. Differential expression of E-cadherin and cytokeratin 19 and net proliferative rate of gingival keratinocytes in oral epithelium in periodontal health and disease. *J Periodontol* 2007;78:2197—202.
7. Avila-Carrasco L, Majano P, Sánchez-Toméro JA, et al. Natural plants compounds as modulators of epithelial-to-mesenchymal transition. *Front Pharmacol* 2019;10:715.
8. Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. *Biochim Biophys Acta* 2000;1477:267—83.
9. Franco C, Patricia HR, Timo S, Claudia B, Marcela H. Matrix metalloproteinases as regulators of periodontal inflammation. *Int J Mol Sci* 2017;18.
10. Checchi V, Maravic T, Bellini P, et al. The role of matrix metalloproteinases in periodontal disease. *Int J Environ Res Publ Health* 2020;17.
11. Ower P. Minimally-invasive non-surgical periodontal therapy. *Dent Update* 2013;40:289—90. 93-5.
12. Rateitschak-Plüss EM, Schwarz JP, Guggenheim R, Düggelin M, Rateitschak KH. Non-surgical periodontal treatment: where are the limits? An SEM study. *J Clin Periodontol* 1992;19:240—4.
13. Lépine G, Caudry S, DiRienzo JM, Ellen RP. Epithelial cell invasion by Actinobacillus actinomycetemcomitans strains from restriction fragment-length polymorphism groups associated with juvenile periodontitis or carrier status. *Oral Microbiol Immunol* 1998;13:341—7.
14. Johnson JD, Chen R, Lenton PA, Zhang G, Hinrichs JE, Rudney JD. Persistence of extracrevicular bacterial reservoirs after treatment of aggressive periodontitis. *J Periodontol* 2008;79:2305—12.

15. Goodson JM, Haffajee A, Socransky SS. Periodontal therapy by local delivery of tetracycline. *J Clin Periodontol* 1979;6: 83—92.
16. Herrera D, Matesanz P, Martín C, Oud V, Feres M, Teughels W. Adjunctive effect of locally delivered antimicrobials in periodontitis therapy. A systematic review and meta-analysis. *J Clin Periodontol* 2020;47:239—56.
17. Cortelli JR, Querido SM, Aquino DR, Ricardo LH, Pallos D. Longitudinal clinical evaluation of adjunct minocycline in the treatment of chronic periodontitis. *J Periodontol* 2006;77: 161—6.
18. Paquette DW, Hanlon A, Lessem J, Williams RC. Clinical relevance of adjunctive minocycline microspheres in patients with chronic periodontitis: secondary analysis of a phase 3 trial. *J Periodontol* 2004;75:531—6.
19. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. *Nat Rev Microbiol* 2012;10:717—25.
20. Castro MML, Duarte NN, Nascimento PC, et al. Antioxidants as adjuvants in periodontitis treatment: a systematic review and meta-analysis. *Oxid Med Cell Longev* 2019;2019:9187978.
21. Chandra RV, Srinivas G, Reddy AA, et al. Locally delivered antioxidant gel as an adjunct to nonsurgical therapy improves measures of oxidative stress and periodontal disease. *J Periodontal Implant Sci* 2013;43:121—9.
22. Chandra RV, Sandhya YP, Nagarajan S, Reddy BH, Naveen A, Murthy KR. Efficacy of lycopene as a locally delivered gel in the treatment of chronic periodontitis: smokers vs nonsmokers. *Quintessence Int* 2012;43:401—11.
23. Belludi SA, Verma S, Banthia R, et al. Effect of lycopene in the treatment of periodontal disease: a clinical study. *J Contemp Dent Pract* 2013;14:1054—9.
24. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: consensus report of workgroup 2 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. *J Periodontol* 2018;89(Suppl 1):S82—173.
25. Silness J, Loe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;22:121—35.
26. Mühlemann HR, Son S. Gingival sulcus bleeding-a leading symptom in initial gingivitis. *Helv Odontol Acta* 1971;15: 107—13.
27. Larsen C, Barendregt DS, Slot DE, Van der Velden U, Van der Weijden F. Probing pressure, a highly undervalued unit of measure in periodontal probing: a systematic review on its effect on probing pocket depth. *J Clin Periodontol* 2009;36: 315—22.
28. Polson AM. The research team, calibration, and quality assurance in clinical trials in periodontics. *Ann Periodontol* 1997;2: 75—82.
29. Giannopoulou C, Cappuyns I, Cancela J, Cionca N, Mombelli A. Effect of photodynamic therapy, diode laser, and deep scaling on cytokine and acute-phase protein levels in gingival crevicular fluid of residual periodontal pockets. *J Periodontol* 2012; 83:1018—27.
30. Preianò M, Falcone D, Maggisano G, et al. Assessment of pre-analytical and analytical variables affecting peptidome profiling of gingival crevicular fluid by MALDI-TOF mass spectrometry. *Clin Chim Acta* 2014;437:120—8.
31. Bogdanovska L, Popovska M, Dimitrovska A, Petkovska R. Development and validation of RP HPLC method for determination of betamethasone dipropionate in gingival crevicular fluid. *Acta Pharm* 2013;63:419—26.
32. Wassall RR, Preshaw PM. Clinical and technical considerations in the analysis of gingival crevicular fluid. *Periodontol 2000* 2016;70:65—79.
33. Hasan A, Palmer RM. A clinical guide to periodontology: pathology of periodontal disease. *Br Dent J* 2014;216:457—61.
34. Ambati M, Rani KR, Reddy PV, Suryaprasanna J, Dasari R, Gireddy H. Evaluation of oxidative stress in chronic periodontitis patients following systemic antioxidant supplementation: a clinical and biochemical study. *J Nat Sci Biol Med* 2017;8:99—103.
35. Sedlacek MJ, Walker C. Antibiotic resistance in an in vitro subgingival biofilm model. *Oral Microbiol Immunol* 2007;22: 333—9.
36. Williams RC, Paquette DW, Offenbacher S, et al. Treatment of periodontitis by local administration of minocycline microspheres: a controlled trial. *J Periodontol* 2001;72:1535—44.
37. Goodson JM, Gunsolley JC, Grossi SG, et al. Minocycline HCl microspheres reduce red-complex bacteria in periodontal disease therapy. *J Periodontol* 2007;78:1568—79.
38. Diamond GA, Kaul S. On reporting of effect size in randomized clinical trials. *Am J Cardiol* 2013;111:613—7.
39. Killeen AC, Harn JA, Erickson LM, Yu F, Reinhardt RA. Local minocycline effect on inflammation and clinical attachment during periodontal maintenance: randomized clinical trial. *J Periodontol* 2016;87:1149—57.
40. Cobb CM. Non-surgical pocket therapy: mechanical. *Ann Periodontol* 1996;1:443—90.
41. Sheets SM, Potempa J, Travis J, Casiano CA, Fletcher HM. Gingipains from Porphyromonas gingivalis W83 induce cell adhesion molecule cleavage and apoptosis in endothelial cells. *Infect Immun* 2005;73:1543—52.
42. Grayson R, Douglas CW, Heath J, Rawlinson A, Evans GS. Activation of human matrix metalloproteinase 2 by gingival crevicular fluid and Porphyromonas gingivalis. *J Clin Periodontol* 2003;30:542—50.
43. Yang IH, Lee J-J, Wu P-C, Kuo H-K, Kuo Y-H, Huang H-M. Oxidative stress enhanced the transforming growth factor-β2-induced epithelial-mesenchymal transition through chemokine ligand 1 on ARPE-19 cell. *Sci Rep* 2020;10:4000.
44. da Silva JC, Muniz F, Oballe HJR, Andrades M, Rösing CK, Cavagni J. The effect of periodontal therapy on oxidative stress biomarkers: a systematic review. *J Clin Periodontol* 2018;45:1222—37.
45. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* 2014; 15:178—96.
46. Reddy NR, Deepa A, Madhu Babu DS, Chandra NS, Subba Reddy CV, Kumar AK. Estimation of tissue inhibitor of matrix metalloproteinase-1 levels in gingival crevicular fluid in periodontal health, disease and after treatment. *J Indian Soc Periodontol* 2014;18:301—5.
47. Ghodpage PS, Kolte RA, Kolte AP, Gupta M. Influence of phase I periodontal therapy on levels of matrix metalloproteinase 1 and tissue inhibitor of metalloproteinase 1. *Saudi Dent J* 2014; 26:171—5.