Research Article

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Locally delivered antioxidant gel as an adjunct to nonsurgical therapy improves measures of oxidative stress and periodontal disease

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Purpose: The present study has two aims; firstly, it attempts to verify the presence of oxidative stress by estimating the reactive oxygen species (ROS) levels in periodontal pockets ≥5 mm as compared to controls. The second aim is to evaluate the effect of lycopene as a locally delivered antioxidant gel on periodontal health and on the gingival crevicular fluid (GCF) levels of 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative injury.
Methods: Thirty-one subjects participated in this study. In the pretreatment phase, the ROS levels in pockets ≥5 mm were measured by flow cytometry. Three sites in each subject were randomly assigned into each of the following experimental groups: sham group, only scaling and root planing (SRP) was done; placebo group, local delivery of placebo gel after SRP; and lycopene group, local delivery of lycopene gel after SRP. Clinical parameters included recording site-specific measures of GCF 8-OHdG, plaque, gingivitis, probing depth, and clinical attachment level.
Results: The gel, when delivered to the sites with oxidative stress, was effective in increasing clinical attachment and in reducing gingival inflammation, probing depth, and 8-OHdG levels as compared to the placebo and sham sites.
Conclusions: From this trial conducted over a period of 6 months, it was found that locally delivered lycopene seems to be effective in reducing the measures of oxidative stress and periodontal disease.

Keywords: Antioxidants, Oxidative stress, Periodontitis.

INTRODUCTION

The term "oxidative stress" describes conditions involving increased reactive oxygen species (ROS) levels [1]. Apart from being implicated in periodontitis [2-4], concepts of oxidative stress are being applied to explain the relationship between periodontitis and various systemic conditions such as metabolic syndrome [5], mitochondrial dysfunction [6], diabetes mellitus [7], and rheumatoid arthritis [8]. Interestingly, all of the associated systemic conditions have therapies focusing on the use of antioxidants to modulate or eliminate the disease [9,10]. Within periodontology itself, the effects of smoking [11], the pathogenesis of *Porphyromonas gingivalis* [12,13], and the possible cytotoxic effects of chlorhexidine digluconate [14] have been explained by applying the principles of oxidative stress.

There seems to be an increasing volume of evidence of an association between oxidative stress and periodontitis [15]. In light of these findings, recognizing the benefits of using antioxidants could aid in the mitigation of oxidative stress, thus

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reducing the damage caused to the periodontal tissues by free radicals. Antioxidants delivered by the diet [16], systemically [17], locally [18], and through a dentifrice [19] have been shown to cause significant improvements in the measures of gingivitis, periodontitis, and oxidative injury. Some antioxidants such as lycopene demonstrate intriguing nonantioxidant properties that are known to have beneficial effects on alveolar bone loss and tooth mobility in subjects with periodontal disease [20]. Conversely, some drugs not traditionally prescribed as antioxidants such as simvastatin [21], melatonin [22], and calcium salts [23] have been shown to prevent inflammatory bone resorption through their latent anti-inflammatory and antioxidant properties.

Among the antioxidants, lycopene is an effective natural antioxidant and exhibits the highest physical quenching rate with singlet oxygen [24]. Systemic administration of lycopene as a monotherapy and as an adjunct to scaling and root planing (SRP) [17] was found to be effective in the management of gingivitis. A recent study has shown that local drug delivery (LDD) of lycopene gel in periodontal pockets is effective in the treatment of chronic periodontitis with a history of smoking [18].

Apart from monitoring ROS production, assaying markers such as 8-hydroxydeoxyguanosine (8-OHdG), which correlates reliably with increased ROS activity during periodontal inflammation, can be used to evaluate the efficacy of antioxidants on oxidant stress [25]. An increase in 8-OHdG can be considered a sign of periodontal damage and the subsequent post-treatment decrease in its levels has been well demonstrated in periodontitis, thus reflecting the status of periodontal health [26].

The present study has two cardinal aims: firstly, it attempts to establish the presence of oxidative stress by estimating the ROS levels in 5 mm periodontal pockets as compared to controls, thus validating the rationale for the use of antioxidant therapy. The second aim is to evaluate the effect of lycopene as a locally delivered antioxidant gel on periodontal health and on the gingival crevicular fluid (GCF) levels of 8-OHdG, a marker of oxidative injury.

MATERIALS AND METHODS

Source of data

31 systemically healthy chronic periodontitis patients (including 20 men) within the age group of 30–55 years (mean age, 33.90±7.66 years) having at least 4 periodontal pockets ≥5 mm with at least 1 pocket in each quadrant showing radiographic evidence of bone loss ≥3 mm were included in the study. The subjects were selected from an initial sample pool of 50 subjects selected from the outpatient section of the Department of Periodontics between July and December, 2011. Smokers and subjects who had received any form of periodontal, antibiotic, or antioxidant therapy within six months of the baseline examination were excluded from the study. Approval from the Institutional Review Board (SVS/2010/3/001) was obtained, and all of the patients provided informed consent. In each subject, 4 teeth with periodontal pockets (one tooth in each quadrant) matched by similar pocket depths were chosen, among which, one site was chosen specifically for the pretreatment phase to serve as a point of comparison for ROS levels.

Study design

Pretreatment phase

The pretreatment phase of the study was designed to establish the presence of oxidative stress, which typically results because of high ROS levels. For ROS estimation, a small portion of the interdental papilla from a pocket with a depth ≥5 mm and from a healthy papilla from the mesial aspect of a third molar (serving as a control) were biopsied.

Cell culture and flow cytometric analysis to assess ROS: Gingival tissues collected aseptically from the periodontal pockets and control sites were placed in a 15 mL sterile tube containing Dulbecco's modified Eagle Medium (DMEM) with 2% gentamicin. The tissue samples were then transferred to a tissue culture dish and rinsed thrice with phosphate buffered saline (PBS) containing 2% gentamicin and then cut into small pieces (1 mm^2). The minced tissues were plated as explants on another 60 mm dish with enriched DMEM containing pyruvic acid, uridine supplemented with 20% fetal calf serum and incubated in a CO_2 incubator at 37°C. When outgrowth of the cells was observed in the cultures, the medium was replaced twice a week until the cells reached confluence.

6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate is a chemically reduced, acetylated form of fluorescein used as an indicator for ROS in cells. For the flow cytometric analysis to assess ROS, gingival fibroblast cells were trypsinized and washed with cold PBS, and 2×10^5 cells were resuspended in l.0 mL DMEM plain media. 0.5 μL of 10 mM carboxy -2′,7′ dichlorofluorescein diacetate (carboxy-H_2DCFDA) (C-400, Invitrogen, Bangalore, India) was added and they were then incubated at 37°C in a dark CO_2 incubator for 15 minutes. The cells were centrifuged down to 1,200 rpm at room temperature, washed thrice with PBS, and dissolved in 500 μL of PBS. The ROS content in the cells was measured by the fluorescence intensity of carboxy-H_2DCFDA excited at 488 nm by using a fluorescence-activated cell sorter (BD FACSelect, BD, San Jose, CA, USA).

Treatment phase

Study design and source of data: The study was designed as a split-mouth, double-blind, randomized controlled clinical trial. Excluding the site used in the pretreatment phase, three teeth in each subject (one in each quadrant) were studied in the treatment phase. In each quadrant, based on a screening examination from the distal aspect of the canine to the mesial aspect of the first molar, one interdental suprabony pocket was chosen for drug delivery and subsequent analysis. The prospective interdental areas were probed buccally and lingually/palatally and the site was considered for the study if the average probing depth (PD) was ≥5 mm. A probing depth threshold of ±1 mm was chosen for all of the sites to be consistent with each other in a given patient. Clinical parameters included noninvasive site-specific measures of the plaque index (PI) [27] and modified gingival index (MGI) [28] at the baseline and at the end of 2, 4, 12, and 24 weeks. The PD, clinical attachment level (CAL), and 8-OHdG levels were the primary outcome variables. The PD and CAL were recorded at the baseline (before SRP) and at the end of 12 and 24 weeks using a University of North Carolina no.15 (UNC-15) color-coded periodontal probe and a custom-made acrylic stent to standardize the probing angulations. 8-OHdG levels were assessed from GCF at the baseline (after SRP) and at the end of 1 and 12 weeks using a commercially available enzyme-linked immunosorbent assay (ELISA) kit.

Sample size: The reduction in 8-OHdG levels was selected as a critical variable for calculating the sample size. Thirty-one patients were required to have an 80% chance (β error) of detecting a significant difference (two-sided 5% level) and a largest difference of 0.70 between groups with a standard deviation of 1, taking into account a 15% rate of attrition of patients. Three subjects were lost during follow-up, limiting the statistical analysis to 28 subjects and 84 teeth (28 sites each in the lycopene, placebo, and sham groups).

Randomization and blinding: Randomization and blinding included computerized generation of the allocation sequence in random permuted blocks (block randomization) and blinded disbursement of medication. Allocation was performed by assigning the block of sites to study groups according to the specified sequence. Based on the sequence, the principal investigator (R.V.C.) selected three sites for each of the following experimental groups: the sham (S) group, only SRP was done; placebo (P) group, local delivery of placebo gel done as an adjunct to SRP; and lycopene (L) group, local delivery of lycopene gel done as an adjunct to SRP. Site preparation and medication delivery was performed by four investigators (G.S., A.A.R., Y.S.H.S.C., and B.H.R.), whereas the relevant readings were recorded by two calibrated investigators (A.N. and S.N.), all of whom were blinded to the nature of the drug placed at the site (lycopene/placebo). The blind was not broken until this clinical trial was completely finished.

Preparation of the 2% lycopene gel

Lycopene (Ibis Chemie International, Bombay, India) was dissolved in a solvent mixture (ethanol: propylene glycol: water in the ratio of 50:30:20). Triethanolamine was added to adjust the pH to above 7.4. The solution was then gelled by adding 8% hydroxypropyl cellulose (HPC) and set aside for 24 hours. The *in situ* gel was prepared to the concentration of approximately 2%. The drug was extensively evaluated for the drug content, viscosity determination, *in vitro* permeation, and stability. The spectrophotometric method reported by Patel et al. [29] was modified for the *in vitro* estimation of the rate of drug release. The *in vitro* release profile showed an initial burst release for the first 24 hours followed by controlled release of lycopene up to 120 hours. The placebo was prepared in the same manner but without the active drug in it.

LDD

SRP was performed at baseline with a piezoelectric ultrasonic scaling unit and a 4R/4L universal scaler until the root surface was considered smooth and clean by the operators. After SRP, GCF was collected and a standardized quantity of 0.1 mL of lycopene (2 mg/0.1 mL) or the placebo was delivered using a specifically modified 1-mL insulin syringe with a blunt end precision tip in the L and P sites, respectively. The subjects were instructed to refrain from aggressively chewing, rinsing, or brushing the test and control sites for 1 week. The experimental gel showed good biological acceptability and with no evidence of discomfort, abscess formation, suppuration, or staining.

Collection of GCF samples

GCF samples were collected 30 minutes after SRP and before the placement of the medications. 0.6-mm filter paper circles (Whatman, Mumbai, India) were placed in the pockets for approximately 30 seconds and fluid seepage from the sulcus was collected. The filter papers were immersed in 1 mL of distilled water immediately after fluid collection. The samples were stored at -30°C and were assayed on the day of collection. Samples were collected at baseline, and at the end of 1 and 12 weeks.

Determination of 8-OHdG in GCF by ELISA

GCF samples were centrifuged at 10,000×g for 10 minutes and the supernatant was used to determine 8-OHdG levels with a commercially available ELISA kit (Highly sensitive

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8-OHdG check, Gentaur Ltd., London, UK). The results were expressed in ng/mL.

Statistical analysis

An analysis based upon knowledge of the expected outcomes in periodontitis patients was used to assess the treatment effect. A site-specific intragroup comparison of PD, CAL, PI, MGI, and 8-OHdG levels between various groups was performed using repeated measures analysis of variance (ANOVA) followed by multiple comparisions using Bonferroni correction. One-way ANOVA followed by the post hoc test was used for intergroup comparison. A *P*-value of <0.01 was considered statistically significant. The data were analyzed using a commercially available software program (SPSS ver. 17.0, SPSS Inc., Chicago, IL, USA).

RESULTS

ROS analysis revealed that there was a 17% increase in ROS production in the gingival fibroblast cells from sites with chronic periodontitis (84.33±3.89) as compared to controls (66.81±3.28) (Fig. 1).

Intragroup comparison of 8-OHdG levels at baseline, 1 week, and 12 weeks showed a statistically significant decrease in the mean 8-OHdG scores across all groups with the differences being more significant from baseline to 1 week and 12 weeks ($P<0.001$). There was a significant reduction in the 8-OHdG levels at the end of the first week when compared to week 12 in the sham and the placebo groups only (Fig. 2).

Intragroup comparison of the PD and CAL at baseline, 12 weeks, and 24 weeks showed a statistically significant reduction in mean scores across all the groups with the differences being more significant from the baseline to 12 and 24 weeks ($P<0.001$). In the lycopene group, however, this reduction was significant ($P=0.001$) when the PD at the end of the 12th week was compared to the score at 24 weeks (Table 1).

Intragroup comparison of PI scores from baseline to 4, 12, and 24 weeks showed a statistically significant reduction in

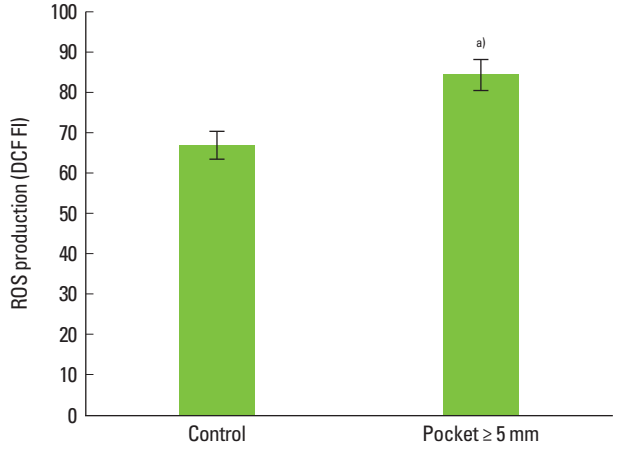

Figure 1. Bar graph representing the percentage of cells showing increased reactive oxygen species (ROS) production compared to controls. Values are reported as fluorescence intensity. Duplicate samples were run to confirm the assay's repeatability, and the mean was taken. DCF FI: dichlorofluorescein fluorescence intensity. [a]Highly significant as compared to the control group.

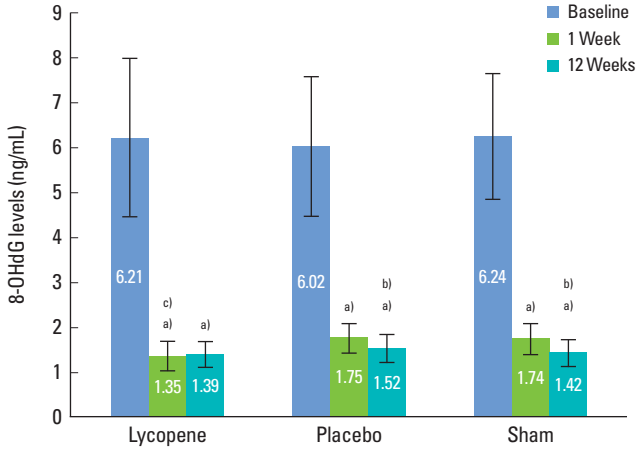

Figure 2. Effects of lycopene, placebo, and sham delivery on the 8-hydroxydeoxyguanosine (8-OHdG) levels at different time periods. [a]Significant intragroup reduction as compared to the baseline levels. [b]Significant intragroup reduction as compared to the levels at 1 week. [c]Intergroup comparison at the first week shows a highly significant reduction in 8-OHdG in the lycopene-treated sites.

Table 1. Intragroup comparision of PD and CAL using repeated measures ANOVA.

Group	PD					CAL				
	Baseline	12 Weeks	24 Weeks	F	*P*-value	Baseline	12 Weeks	24 Weeks	F	*P*-value
Lycopene	6.07±1.07	4.40±1.04[a]	3.62±1.04[a],[b]	127.97	0.001	6.55±1.15	4.62±1.07[a]	3.77±1.01[a],[b]	118.59	0.001
Placebo	5.66±1.17	4.55±1.18[a]	4.51±1.22[a]	66.31	0.001	5.85±1.32	4.81±1.14[a]	4.77±1.25[a]	49.27	0.001
Sham	5.88±1.28	5.03±1.28[a]	4.92±1.35[a]	40.26	0.001	6.22±1.28	5.48±1.22[a]	5.14±1.45[a]	23.13	0.001

Values are presented as mean±standard deviation.
PD: probing depth, CAL: clinical attachment level, ANOVA: analysis of variance.
[a]Significant as compared with baseline using multiple comparison test. [b]Significant as compared with the levels at 1 week using multiple comparison test.

all the groups ($P<0.001$). In the lycopene group, however, this reduction was significant ($P=0.001$) when the PI at the end of the second week was compared to the baseline score (Table 2). Intragroup comparison of the MGI scores from baseline to 4, 12, and 24 weeks showed a statistically significant reduction in all of the groups ($P<0.001$). In addition, in all of the groups, there was no significant reduction when the MGI score at the end of the second week was compared to the baseline score (Table 1).

Table 2. Intergroup comparison at different time based intervals using ANOVA.

Group	Lycopene vs. placebo vs. sham	
	F	*P*-value
PD		
Baseline	0.80	0.450
12 Weeks	2.11	0.120
24 Weeks	8.05	0.001[b]
CAL		
Baseline	2.21	0.120
12 Weeks	4.10	0.020[a]
24 Weeks	8.62	0.001[b]
PI		
Baseline	0.28	0.750
2 Weeks	6.05	0.001[b]
4 Weeks	3.88	0.020[a]
12 Weeks	3.99	0.020[a]
24 Weeks	0.25	0.770
MGI		
Baseline	0.02	0.970
2 Weeks	4.24	0.010[a]
4 Weeks	33.80	0.001[b]
12 Weeks	0.73	0.480
24 Weeks	9.76	0.001[b]
8-OHdG		
Baseline	0.14	0.860
1 Week	12.94	0.001[b]
12 Weeks	1.29	0.270

ANOVA: analysis of variance, PD: probing depth, CAL: clinical attachment level, PI: plaque index, MGI: modified gingival index, 8-OHdG: 8-hydroxydeoxyguanosine.
[a]Significant. [b]Highly significant.

Intergroup comparison of the three test groups were performed at different time-based intervals for 8-OHdG, PD, CAL, PI, and MGI. For the 8-OHdG levels, lycopene showed a highly significant reduction in scores at the first week when compared to the other two groups ($P<0.001$). For PD, a highly significant ($P<0.001$) decrease in pocket depth was seen in the lycopene group at 24 weeks when compared to the sham and placebo groups. In the comparison of CALs among the three study groups, the lycopene group showed a significant gain in the clinical attachment at the end of week 12 ($P=0.02$) as compared to the other two groups. However, the gain was highly significant ($P<0.001$) in the lycopene group at the end of 24 weeks. Likewise, the lycopene group showed a highly significant reduction in the mean plaque scores at the end of two weeks ($P<0.001$) and a significant reduction ($P<0.01$) in the MPI at the end of four weeks and twelve weeks when compared to the other two groups. Finally, with regard to the MGI, a significant reduction in the mean gingival scores was seen in the lycopene group at the end of two weeks ($P<0.01$) and a highly significant reduction at the end of four weeks ($P<0.001$) (Table 3).

Pairwise comparison of the lycopene versus placebo group for the different variables showed that the lycopene group demonstrated a highly significant reduction in 8-OHdG levels at week 1 ($P<0.001$), a significant and a highly significant reduction in MGI scores at week 2 ($P=0.04$) and 4, respectively, with a significant reduction in the PI at weeks 2, 4, and 12. There was a significant reduction in the pocket depth at 24 weeks ($P=0.02$) and a significant gain in the CAL at 24 weeks ($P=0.01$) (Tables 2, 4, 5).

Pairwise comparison of the lycopene versus sham group for the different variables showed that the lycopene group demonstrated a highly significant reduction in 8-OHdG levels at week 1 ($P<0.001$), a significant reduction in the MGI score at week 2 ($P=0.04$), and a highly significant reduction in the MGI scores at weeks 4 and 24 ($P<0.001$), with a significant reduction in mean plaque scores at 2, 4, and 12 weeks. There was also a highly significant reduction in PD and CAL

Table 3. Intragroup comparision of PI and MGI scores using repeated measures ANOVA.

Group	PI							MGI						
	Baseline	2 Weeks	4 Weeks	12 Weeks	24 Weeks	F	*P*-value	Baseline	2 Weeks	4 Weeks	12 Weeks	24 Weeks	F	*P*-value
Lycopene	3.55±0.57	2.73±0.72[a]	2.08±0.78[a]	1.79±0.55[a]	1.68±0.60[a]	42.54	0.001	2.92±0.43	2.66±0.76	0.78±0.57[a]	0.81±0.47[a]	0.79±0.47[a]	117.07	0.001
Placebo	3.68±0.60	3.20±0.60	2.55±0.68[a]	1.58±0.49[a]	1.73±0.54[a]	68.19	0.001	2.93±0.36	3.17±0.76	0.89±0.50[a]	0.91±0.65[a]	0.90±0.62[a]	91.33	0.001
Sham	3.67±0.59	3.34±0.75	2.55±0.68[a]	2.23±0.77[a]	1.77±0.46[a]	47.35	0.001	2.94±0.36	3.20±0.81	1.76±0.48[a]	0.99±0.61[a]	1.36±0.37[a]	84.98	0.001

Values are presented as mean±standard deviation.
PI: plaque index, MGI: modified gingival index, ANOVA: analysis of variance.
[a]Significant as compared with baseline using multiple comparison test.

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at 24 weeks and a significant reduction in CAL at 12 weeks (P=0.02) (Tables 2, 4, 5).

Pairwise comparison of the placebo versus sham group for the different variables showed there was clinically significant difference only in the mean gingival index scores at the end of 24 weeks, with the placebo group showing a highly significant reduction as compared to the sham group (P<0.001) (Tables 2, 4, 5).

Table 4. Post hoc pairwise comparison of 8-OHdG levels, PD and CAL using multiple comparison test.

Group	Mean difference	*P*-value
8-OHdG		
Baseline		
Lycopene vs. placebo	0.18	0.900
Lycopene vs. sham	–0.02	0.980
Placebo vs. sham	–0.21	0.870
1 Week		
Lycopene vs. placebo	–0.39	0.001[b]
Lycopene vs. sham	–0.38	0.001[b]
Placebo vs. sham	0.00	0.990
12 Weeks		
Lycopene vs. placebo	–0.12	0.277
Lycopene vs. sham	–0.02	0.930
Placebo vs. sham	0.09	0.460
PD		
Baseline		
Lycopene vs. placebo	0.40	0.410
Lycopene vs. sham	0.18	0.830
Placebo vs. sham	–0.22	0.760
12 Weeks		
Lycopene vs. placebo	–0.14	0.880
Lycopene vs. sham	–0.62	0.120
Placebo vs. sham	–0.48	0.290
24 Weeks		
Lycopene vs. placebo	–0.88	0.020[a]
Lycopene vs. sham	–1.29	0.001[b]
Placebo vs. sham	–0.40	0.430
CAL		
Baseline		
Lycopene vs. placebo	0.70	0.100
Lycopene vs. sham	0.33	0.590
Placebo vs. sham	–0.37	0.520
12 Weeks		
Lycopene vs. placebo	–0.18	0.820
Lycopene vs. sham	–0.85	0.020[a]
Placebo vs. sham	–0.66	0.100
24 Weeks		
Lycopene vs. placebo	–1.00	0.010[a]
Lycopene vs. sham	–1.37	0.001[b]
Placebo vs. sham	–0.37	0.520

8-OHdG: 8-hydroxydeoxyguanosine, PD: probing depth, CAL: clinical attachment level.
[a]Significant. [b]Highly significant.

Table 5. Post hoc pairwise comparison of PI and MGI scores using multiple comparison test.

Group	Mean difference	*P*-value
PI		
Baseline		
Lycopene vs. placebo	0.11	0.770
Lycopene vs. sham	0.10	0.780
Placebo vs. sham	0.01	1.000
2 Weeks		
Lycopene vs. placebo	–0.62	0.010[a]
Lycopene vs. sham	–0.62	0.010[a]
Placebo vs. sham	0.00	1.000
4 Weeks		
Lycopene vs. placebo	–0.47	0.040[a]
Lycopene vs. sham	–0.47	0.040[a]
Placebo vs. sham	0.00	1.000
12 Weeks		
Lycopene vs. placebo	–0.46	0.040[a]
Lycopene vs. sham	–0.46	0.040[a]
Placebo vs. sham	0.00	1.000
24 Weeks		
Lycopene vs. placebo	–0.08	0.810
Lycopene vs. sham	–0.05	0.810
Placebo vs. sham	0.00	1.000
MGI		
Baseline		
Lycopene vs. placebo	–0.01	0.990
Lycopene vs. sham	–0.02	0.970
Placebo vs. sham	–0.01	0.990
2 Weeks		
Lycopene vs. placebo	–0.52	0.040[a]
Lycopene vs. sham	–0.56	0.020[a]
Placebo vs. sham	–0.03	0.980
4 Weeks		
Lycopene vs. placebo	–1.08	0.001[b]
Lycopene vs. sham	–0.94	0.001[b]
Placebo vs. sham	0.13	0.620
12 Weeks		
Lycopene vs. placebo	–0.10	0.780
Lycopene vs. sham	–0.18	0.450
Placebo vs. sham	–0.08	0.840
24 Weeks		
Lycopene vs. placebo	–0.11	0.660
Lycopene vs. sham	–0.55	0.001[b]
Placebo vs. sham	–0.44	0.004[b]

PI: plaque index, MGI: modified gingival index.
[a]Significant. [b]Highly significant.

DISCUSSION

Antioxidant therapy is believed to be effective in periodontitis [2]. However, studies exploring the role of antioxidants have shown mixed results [30-32]. While the aims are the same, this study sought to improve on the methodology used in the previous studies, which found that antioxidant gels were effective in decreasing PDs and increasing CALs.

In spite of being a placebo-controlled trial, the study faced two unique situations that necessitated the use of a sham site. First, in a pilot study preceding the main trial, an unexpected observation was the subject's ability to identify sites under investigation and second, the vehicle used in this study, HPC (which is a cellulose derivative) is also known to have a very minor and nonsignificant antioxidant activity of its own. The possible influence of the Hawthorne effect in periodontal intervention studies is well known [33] and unintended but overzealous plaque control around the study sites can be expected. Thus the need to further "mask" the study and placebo sites by the introduction of a "sham" site was an attempt at having a clinical setup to negate this effect. No evidence of a Hawthorne effect was observed in this study as there were no significant differences in intragroup measures of plaque and gingivitis during different time periods. HPC has been utilized as a vehicle in periodontics before [34], but its latent antioxidant activity has probably not been investigated before primarily because it has been used to deliver antibiotics, antiseptics, and growth factors. Both the lycopene and placebo syringes contained HPC and the introduction of the sham site was also an attempt in this study to separate and identify the actual clinical effect from artifacts caused by any physical or chemical properties attributable to the vehicle. However, contrary to what was anticipated, there was no significant inter-group variation in the 8-OHdG levels between the sham and placebo groups while the lycopene treated sites showed a significant reduction in 8-OHdG levels as compared to the placebo. Interestingly, the sham sites demonstrated a greater reduction in 8-OHdG levels at week 1 than the placebo sites (4.5 ng/mL vs. 4.27 ng/mL), and looking at the endpoints, HPC did not seem to have a positive effect on oxidative damage in this study.

In this study, lycopene was delivered to sites demonstrating oxidative stress as evidenced by increased ROS levels in pockets with similar PD. A positive relationship exists between the ROS level and 8-OHdG level [35], as 8-OHdG itself is a product of ROS-mediated oxidative damage to DNA in various cells. Lycopene is known to act as a modulator of intracellular ROS activity [36] and while this study does not investigate this relationship, it can be speculated that lycopene can moderate levels of oxidative stress to a level where ROS can have beneficial effects to the periodontal tissue (hormesis effect). In this study, reduced levels of 8-OHdG present a picture of a balance-tilt towards reduction in ROS production and oxidative stress [35,36], thus providing indirect evidence of the hormetic effect of lycopene.

In this investigation, we have considered the 8-OHdG levels in GCF. To the best of our knowledge, this is only the third study to have assayed 8-OHdG levels directly from the GCF [18,37]. In the present study, all the groups showed highly significant reduction in GCF levels of 8-OHdG from baseline to the end of the assay period and the results are in agreement with the previous studies, in which periodontal treatment with or without lycopene therapy has been associated with a decrease in 8-OHdG levels [17,18]. In the lycopene treated sites, the 8-OHdG levels exhibited a small nonsignificant increase from the first to the twelfth week. This intriguing increase in the 8-OHdG levels was also reported in a previous study, which is probably indicative of decreasing lycopene action [18]. In addition, pro-oxidant agents generally show a rebound effect in their activity after suppression by antioxidants, which might have also caused this trend toward a rising 8-OHdG level [17,18]

Lycopene, commonly delivered as a gel, shows acceptable kinetics in the mucosal surfaces of the human oral cavity [18]. This drug reaches a peak concentration within 15–36 hours and returns to baseline levels between approximately 80 and 104 hours. When compared to the placebo and the sham sites, lycopene-treated sites showed a significant reduction in PDs and attachment loss levels, but only during the 12–24 week period. Though the reduction in the 8-OHdG levels and the improvement in CAL and PD were significant in different time frames, the initial reduction in the oxidative injury can be considered a critical contributor towards the gain in the CAL and reduction in the PD. The evidence can be listed chronologically as follows: first, the efficacy of lycopene in lowering oxidative stress in periodontal pockets; second, as evident from the 8-OHdG levels, lycopene exerts its maximum effect within the first week and is able to maintain its therapeutic action for longer periods of time; and third, SRP alone can contribute to significant improvement in periodontal health and oxidative stress markers, and the use of lycopene as an adjunct to SRP had further reduced the PD by the end of the study period. The lycopene-treated sites showed a significant reduction in gingivitis and plaque scores as well. Lycopene is known to exhibit an inhibitory effect on bacterial colonization [12,13] and the results seem to suggest that lycopene can be used as an adjunct to SRP in the control of gingivitis.

The current study has limitations that need to be considered when interpreting the results. In the pretreatment phase, rath-

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er than assessing the GCF for markers of oxidant damage, we chose to assay the ROS levels from a fibroblast cell-culture obtained from the papilla, as it accurately reflects the presence of oxidative injury in the lateral pocket wall [38]. In the treatment phase, however, 8-OHdG levels from the GCF were assayed, as the results of ROS levels cannot be used independently to ascertain the efficacy of antioxidant gel for several reasons: First, the extent to which ROS production influences the initiation and progression of periodontal diseases is still unknown [3], second, fluctuating antioxidant levels may also influence the accuracy of the intracellular 2,7-dichlorofluorescin oxidation test used to measure the production of ROS in this study [39], and third, the DCF assay is also more highly sensitive to levels of H_2O_2 than other ROS which makes it unsuitable for evaluating the effects of lycopene, which primarily shows its action on singlet oxygen [38,40]. Because there was strong evidence of oxidative stress in the pretreatment phase, a less stringent criterion was employed to evaluate the reduction in oxidative injury during the treatment phase of the study. Another limitation was that 8-OHdG levels were compared only among periodontally involved sites during periodontal therapy without the inclusion of a periodontally healthy control group. However, we feel that this limitation is less concerning, as the post-treatment 8-OHdG levels in all groups were consistent with a previous study describing [18] the levels of 8-OHdG in periodontally healthy sites.

In conclusion, the analyses of the results clearly illustrate the efficacy of lycopene in improving measures of oxidative stress and periodontal disease when delivered into sites with profound oxidative activity. Further studies are required to determine the long-term effects of locally delivered antioxidants on various clinical and surrogate endpoints of interest in the field of periodontics.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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