Clinical and Biochemical Assessment of Lycopene Gel Combined With Nanohydroxyapatite Graft in Treatment of Grade II Furcation Defects: A Randomized Controlled Clinical Study

Dalia Shawky Gaber,[A] Nevine Hassan Kheir El Din,[B] Hamdy Ahmed Nassar,[C] Mostafa Saad El-Din Ashmawy,[B] Ola Mohamed Ezzatt[B]

[A]Department of Oral Medicine, Periodontology, Oral Diagnosis, and Oral Radiology, Faculty of Dental Medicine, October 6 University, Giza, Egypt.

[B]Department of Oral Medicine, Periodontology, Oral Diagnosis, and Oral Radiology, Faculty of Dental Medicine, Ain Shams University, Cairo, Egypt.

[C]Department of Oral Medicine, Periodontology, Oral Diagnosis, and Oral Radiology, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt.

Accepted for publication: December 18, 2021

Abstract

Background: This study aimed to evaluate the effects of lycopene gel, as a natural antioxidant, mixed with a nanohydroxyapatite graft (NHG) covered by an occlusive resorbable collagen membrane (CM) in the surgical treatment of grade II furcation defects and on the gingival crevicular fluid (GCF) levels of 8-hydroxydeoxyguanosine (8-OHdG), as a marker of oxidative injury. **Methods:** In this randomized controlled clinical study a total of 24 patients with grade II furcation defects were randomly assigned into three equal groups. Furcation defects in group I were managed with lycopene gel mixed with NHG and CM, group II with NHG and CM, and group III with open flap debridement only. Site-specific changes in clinical parameters including probing depth (PD), vertical clinical attachment level (VCAL), horizontal clinical attachment level (HCAL), radiographic maximum vertical depth (MAX V), and maximum horizontal depth (MAX H) were measured at baseline and six months postoperatively. Gingival crevicular fluid levels of 8-OHdG were analyzed using enzyme-linked immunosorbent assay (ELISA) at baseline, one week, and three months. **Results:** Surgical management of grade II furcation defects resulted in a significant reduction in PD and 8-OHdG levels and a gain in CAL, MAX V, and MAX H in all groups. The differences between lycopene treated sites compared to NHG and CM alone were not significant at six months but demonstrated significantly superior clinical parameters compared to open flap debridement alone. **Conclusion:** Lycopene does not confer a benefit when combined with NHG in the surgical treatment of grade II furcation defects.

Keywords: Furcation involvement; lycopene; nanohydroxyapatite; periodontal regeneration; 8-hydroxydeoxyguanosine; collagen membrane

Introduction

Periodontitis is an inflammatory condition affecting the gingiva, periodontal ligament, alveolar bone, and cementum.[1] The primary causative factors for periodontitis are gram-negative anaerobic or facultative anaerobic bacteria within the subgingival biofilm. Thus, periodontal therapy aims to remove bacteria using conventional periodontal pocket debridement (PPD). However, most of the periodontal tissue damage occurs due to the abnormal host response to pathogenic bacteria including overproduction of

reactive oxygen species (ROS) by host defense cells.[2] The imbalance between ROS production and the antioxidant defense system might lead to the generation of oxidative stress, which plays an important role in the pathogenesis of periodontal disease.[3,4]

The furcation area poses an additional challenge for the clinician to manage. This is because the unfavorable complex morphology and restricted-access area that characterizes furcation defects limit not only the efficacy of nonsurgical and surgical therapies, but also the patient's self-performed plaque control.[5] The American Academy of Periodontology (AAP) concluded in its regeneration workshop on furcation defects that regeneration is a viable treatment option for molars with class II defects and recommended this approach to be considered before other treatments.[6]

Markers such as 8-hydroxydeoxyguanosine (8-OHdG), which correlates reliably with increased ROS production and activity during periodontal inflammation, can be used to evaluate the efficacy of antioxidants on oxidant stress.[7] An increase in 8-OHdG can be considered a sign of periodontal destruction and the subsequent post-treatment decrease in its levels has been well demonstrated in periodontitis, thus reflecting the status of periodontal health.[8]

Lycopene is one of the most effective antioxidants found in red-colored fruits such as tomatoes and exhibits the highest quenching rate with singlet oxygen.[9] Lycopene scavenges a large spectrum of free radicals including superoxide, hydrogen peroxide, and hydroxyl radicals. Chandra et al. reported a significant improvement in patients with gingivitis and periodontitis following systemic oral administration of lycopene soft gels and the local application of lycopene gel as a monotherapy and as an adjunctive therapy along with PPD.[10-12] However, there is a lack of evidence to support the use of lycopene locally with periodontal surgery.

Considering the previously mentioned properties of lycopene, we hypothesized that it would have a promising effect on the regeneration of furcation defects. To the best of the authors' knowledge, this is the first study to clinically investigate the possible adjunctive use of lycopene in the surgical management of grade II furcation defects. The study also evaluated the antioxidant effect of lycopene through the measurement of gingival crevicular fluid (GCF) levels of 8-OHdG.

Materials and Methods

I. Patient Selection

Thirty-two systemically healthy patients, based on a medical health questionnaire modified from *Burket's Oral Medicine*, were selected from the outpatient clinic of the Periodontology Department of the Ain Shams University Faculty of Dental Medicine and the October 6 University Faculty of Dental Medicine.[13] Subjects recruited in this 3-arm randomized controlled, assessor-blinded clinical trial had localized stage III grade B periodontitis according to the most recent AAP classification.[14]

Periodontitis affected patients were randomly allocated using computer assessed randomization[a] into three study groups of eight patients each. Group I included eight grade II furcation sites managed by open flap surgical debridement with lycopene gel[b] mixed with a nanohydroxyapatite graft (NHG)[c] covered by an occlusive resorbable collagen membrane[d] and was considered the test group. Group II included eight grade II furcation sites managed by open flap surgical debridement with a placebo gel mixed with NHG covered by an occlusive resorbable collagen membrane and was considered the positive control group. Group III included eight grade II furcation sites managed only by open flap debridement and was considered the negative control group.

The affected sites were selected for the study if grade II furcation involvement of the affected molar teeth evidenced

[a] Random Allocation Software 2.0
[b] Nanjing Zelang Medical Technology Co., Ltd., Nanjing, China
[c] Nanostreams Company-NS0012, 6th of October City, Egypt
[d] Hypro-Sorb® F, Bioimplon GmbH, Giessen, Germany

radiographically (using periapical radiographs for preliminary diagnosis) and clinically with a probing depth (PD) ≥4 mm, clinical attachment level (CAL) ≥5 mm, and horizontal probe penetration ≥3 mm one month following phase I periodontal therapy. Subjects with a history of periodontal surgery or administration of antimicrobial drugs or over the counter antioxidants within the three months prior to the baseline examination were excluded. In addition, subjects who reported any side effects or drug allergies from antioxidants and those with unacceptable oral hygiene upon re-evaluation of phase I therapy were also excluded. The study protocol was reviewed and approved by the research ethics committee of the Ain Shams University Faculty of Dental Medicine (FDASU-REC) in April 2016. Patients signed an informed consent and were permitted to withdraw at any time without penalty or loss of any benefits. The study protocol was registered and identified as NCT03203745 at the U.S. National Institutes of Health Clinical Trials Registry.

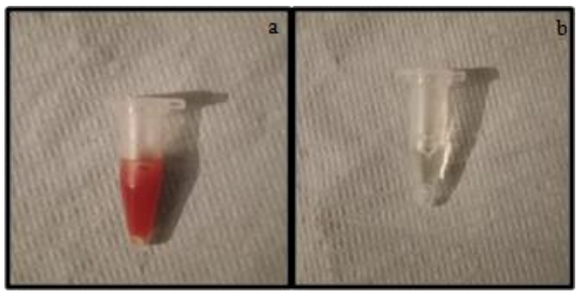
II. Interventions

Initial cause-related therapy consisted of a single session of full mouth PPD performed at baseline using an ultrasonic scaling unit[e], a 4R-4L universal curette, and Gracey *Mini Five* curettes[f] until the root surface was clinically smooth and free of debris.

Preparation of Lycopene Gel and Placebo Gel

Lycopene was purchased in powder form (10 gm) and was divided into 10 Eppendorf tubes, each containing 1 gm, and was microbiologically tested to ensure its safety (negative microbial test) and a heavy metal test was conducted (0.13%) to avoid taste changes. Lycopene gel was then dissolved in a solvent mixture of ethanol: propylene glycol: water in a ratio of 50:30:20. Triethanolamine was added to adjust the pH to above 7.4. The solution was gelled by adding 8% hydroxypropyl cellulose (HPC) and was set aside for 24 hours. The in situ gel was prepared to a concentration of approximately 2%. The lycopene gel was red in color, odorless, and viscous for easy handling, manipulation, and adaptation of the mixture (gel and graft) to the defect. The placebo gel was prepared in the same manner without the active drug and it was transparent and odorless (Figures 1A and 1B).

Figure 1.



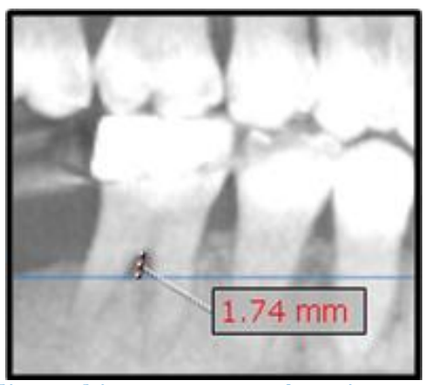
A. Lycopene gel; B. Placebo gel

III. Presurgical Procedure

Presurgical Radiographic Measurements

Prior to any clinical measurements, a cone beam computed tomography (CBCT) image of the site to be operated on was obtained. The radiographic maximum vertical depth (MAX V) was recorded in the sagittal view by measuring the distance from the furcation entrance to the base of the defect in the vertical direction (Figure 2).[15] The radiographic maximum horizontal depth (MAX H) was recorded in the axial view by drawing a line from the most buccal end of one root to the other root followed by a perpendicular line from the center of the first line to the bone trabeculae start or crest (Figure 3).[16]

Figure 2.

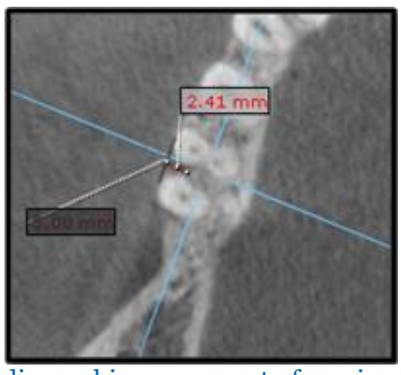


Radiographic assessment of maximum vertical depth of the furcation (MAX V)

[e] Woodpecker UDS-K LED Ultrasonic Scaler

[f] Hu-Friedy Mfg. Co., LLC, Chicago, IL, USA

Figure 3.



Radiographic assessment of maximum horizontal depth of the furcation (MAX H)

Presurgical Clinical Examination

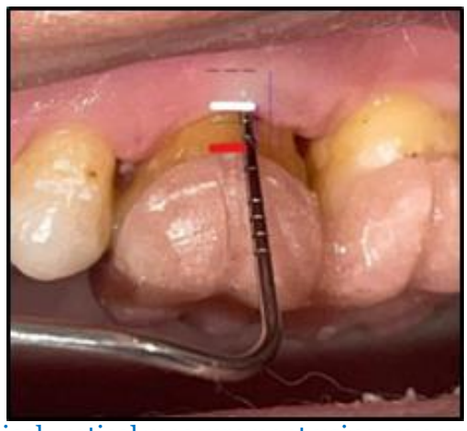
Baseline clinical parameters were recorded and included PD, plaque index (PI), gingival index (GI), vertical clinical attachment level (VCAL), and horizontal clinical attachment level (HCAL).[17,18] The measurements were taken using the University of Michigan "O" probe with Williams markings and the Nabers probe.[g] Clinical parameters were documented on the day of the surgical procedure immediately prior to surgery (D.S.G.).

Custom-made acrylic stents were fabricated to standardize the probe position and angulations (Figure 4). The occlusal stents were made with cold cure acrylic resin on a cast model obtained from an alginate impression and a groove was made in the stent in relation to each involved tooth. Probing depth was measured by subtracting the stent level (distance from the stent margin to the gingival margin) from the relative pocket depth (distance from the stent margin to the depth of the pocket).[19] The vertical clinical attachment level was measured by subtracting the distance from the stent margin to the cementoenamel junction (CEJ) from the relative pocket depth.[19] The horizontal clinical attachment level was measured with a Nabers probe. The probe was penetrated until resistance was felt and the distance from the furcation entrance to the depth was recorded (Figure 5).[16]

GCF Collection and Enzyme-Linked Immunosorbent Assay (ELISA)

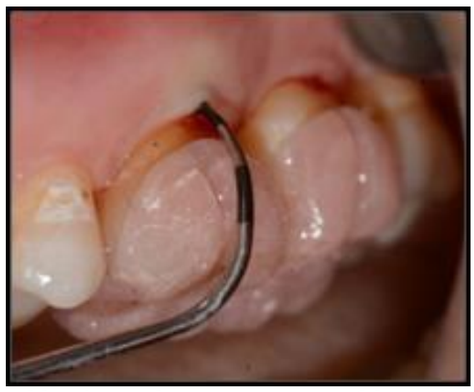
The site of the tooth was isolated with cotton rolls and a thin plaque film if present was removed manually using clean gauze.[20] The site was then dried and the GCF was collected using filter paper[h] which was inserted into the depth of the pocket until minimal resistance was felt and was then left in the site for 30 seconds. The filter paper was subsequently removed from the pocket and inspected for any sign of contamination with either saliva or blood. Contaminated papers were discarded and the procedure was repeated after 30 minutes; otherwise, the samples were transferred to a pre-weighed Eppendorf tube that contained 200 ml of phosphate-buffered saline[i] and stored at -30°C till the time of processing.[11]

Figure 4.



Clinical vertical measurement using an acrylic stent; the red line is the CEJ, the white line is the gingival margin, the green line represents the stent margin, and the non-continuous line represents the base of the pocket.

Figure 5.



Clinical horizontal measurement of furcation using Nabers probe

IV. Surgical Phase

Following administration of local anesthesia and after rinsing with 0.12% chlorhexidine

[g] Hu-Friedy Mfg. Co., LLC, Chicago, IL, USA
[h] Whatman™
[i] Euroclone®, Milan, Italy

gluconate[j] a mucoperiosteal flap was reflected and thorough debridement was carried out (Figure 6A). The nanohydroxyapatite graft and resorbable collagen membrane were incorporated into the clean site either with lycopene gel in group I or with a placebo in group II and only open flap debridement was carried out in group III (Figures 6B and 6C). The flap was coronally advanced and secured with direct interrupted sutures to obtain primary closure with polypropylene monofilament, non-absorbable sutures[k] (Figure 6D).

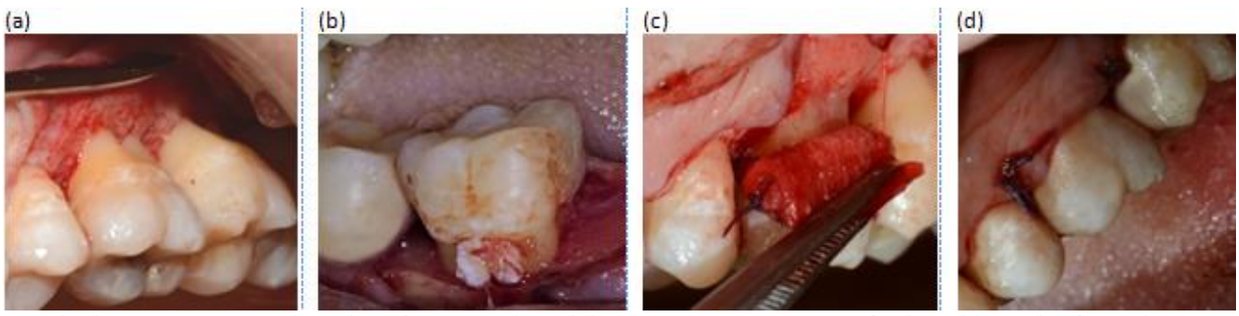
V. Postsurgical

Patients were instructed to not brush the surgical site for a period of three weeks and to instead gently wipe the area with a cotton pellet dampened with saline and to rinse with 0.12% chlorhexidine gluconate twice daily for a period of three weeks starting 24 hours postoperatively. Postoperative medications including amoxicillin clavulanate 1 gm (one capsule every 12 hours for five days) and ibuprofen 600 mg (one tablet three times daily after meals for four days) were prescribed.[16] Sutures were removed ten days postoperatively. All clinical parameters were recorded again six months postoperatively and all radiographic parameters were again recorded nine months postoperatively. Patients were recalled after one week and three months to collect GCF samples for biochemical analysis in order to evaluate the levels of 8-OHdG which was used as a biomarker.

VI. Statistical Analysis

Based on a study conducted by Chandra et al., sample size was estimated assuming alpha error = 5% and study power = 80% and assuming effect size = 1.09 on comparing means of clinical attachment loss between the study and control group.[12] The minimum sample size was calculated, using computer software,[l] to be seven per group which was increased to eight assuming dropouts. All data were collected, tabulated, and statistically analyzed using computer software.[m] The difference between the two groups was statistically analyzed using an independent sample with the Student's t-test and paired t-test. The F value of an analysis of variance (ANOVA) was calculated to compare between the three groups. All levels of significance were set at $P<0.05$.

Figure 6.



Surgical steps: A. Furcation site after complete debridement; B. NHA graft placed into the furcation defect; C. Adaptation of collagen membrane over the furcation defect; D. Sutured flap

Results

The study included nine males and 15 females, with an age range from 34-54 years (mean age: 43 ±6.4 years). At baseline, there was no statistically significant difference between the three groups with regards to PD, VCAL, HCAL, MAX V, and MAX H mean values. At six months, there was a reduction in all clinical and radiographic parameters which was statistically significant in each group. By comparing the three groups to each other, mean PD, VCAL, HCAL, MAX V, and MAX H for groups I and II at six months did not show a statistically significant difference, although group III's parameters were significantly greater than group I and group II. Furthermore, significant reductions in furcation depths were demonstrated horizontally by 1.69 ±0.41 mm in group I, 1.73 ±0.48 mm in group II, and 0.64 ±0.66 mm in group III, and vertically by

[j] Hexitol 0.12%, Arab Drug Company (ADCO), Cairo, Egypt
[k] Prolene®, 4-0, 5-0, Ethicon, Raritan, NJ, USA
[l] G*Power 3.1.9.7, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
[m] IBM SPSS Statistics 21, Armonk, New York, USA

1.11 ±0.34 mm in group I, 1.25 ±0.37 mm in group II, and 0.62 ±0.54 mm in group III (Table 1). Biochemical analysis revealed that there was no statistically significant difference between the three groups with regards to the mean value of 8-OHdG, which decreased significantly in all groups (Table 2).

Table 1: ANOVA and mean changes in PD, VCAL, HCAL, MAX V, and MAX H in different treatment groups (mean± SD)

Clinical or Radiographic Parameter	Follow-up	Group I (n=8)	Group II (n=8)	Group III (n=8)	P-Value
PD	Base Line	4.75±1.48	4.50±1.06	4.87±0.83	0.808 ns
	6 Months	2.62±0.51[a]	2.75±0.70[a]	3.87±0.35[ab]	0.000***
VCAL	Base Line	5.00±1.06	5.37±1.50	5.10±0.64	0.797ns
	6 Months	3.37±0.51[a]	3.12±0.35[a]	4.37±0.51[ab]	0.000***
HCAL	Base Line	11.00±1.06	11.37±0.91	10.87±0.83	0.554ns
	6 Months	7.87±0.35[a]	8.00±0.53[a]	10.12±0.64[ab]	0.000*
MAX V	Base Line	1.72±0.25	1.84±0.23	1.95±0.48	0.421 ns
	6 Months	0.61±0.09[a]	0.59±0.18[a]	1.33±0.32[ab]	0.000*
MAX H	Base Line	2.25±0.20	2.53±0.31	2.09±0.48	0.063ns
	6 Months	0.56±0.29[a]	0.80±0.36[a]	1.45±0.69[ab]	0.001**

Significance level: *P<0.05, **P<0.01, ***P<0.001; ns: non-significant; similar superscripts in same row are indicative of non-significant differences

Table 2. ANOVA and mean 8-OHdG levels in GCF among study groups (mean± SD)

Time of Assessment	Group I (n=8)	Group II (n=8)	Group III (n=8)	P- Value
Base Line	23.05±2.29	22.63±1.55	21.92±1.83	0.705 ns
1 Week	11.96±1.41	13.16±1.36	12.86±2.14	1.108 ns
3 Months	12.58±1.06	11.23±1.38	11.11±1.97	2.306 ns
P-Value	0.000***	0.000***	0.000***	

Significance level: ***P<0.001; ns: non-significant

Discussion

In the present study, the reduction in oxidative stress was reflected by a decrease in PD and CAL. The results of the current study are in agreement with multiple studies that evaluated oxidative stress in periodontally diseased individuals and with studies that evaluated the efficacy of locally delivered lycopene gel in chronic periodontitis.[4,11,12,21-22] In our study, postoperative follow-up at six months showed a significant reduction in PD and CAL in all treatment groups and these findings were in accordance with the outcomes of several studies.[11,12,23] Thus, we concluded that a reduction in PD and CAL can be obtained through appropriate PPD.[24,25] In the present study, lycopene treated sites compared to open flap debridement alone showed a significant reduction in PD and CAL which was in agreement with several studies that evaluated lycopene locally.[11,12,23]

Some studies that evaluated lycopene systemically, showed nonsignificant results when compared to the placebo group which was not in agreement with the previously mentioned studies.[26,27] Our explanation for the nonsignificant differences witnessed in our study with regards to the lycopene and placebo groups, is that lycopene exerts its maximum effect during the first week with a gradual decrease in its therapeutic action for a period of three months.[12] As the initial reduction in oxidative injury can be considered a critical contributor towards the reduction in PD and CAL, and lycopene's effect on oxidative injury decreases with time, its beneficial effects on clinical parameters are ultimately negated**.** Thus, 2% lycopene gel can be employed for controlled local delivery for only seven days in subgingival areas[11]. 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.[10,11] The

second explanation is that the regenerative technique, which included NHG and a collagen membrane, plays a role in PD and CAL reduction, as well as a decrease in the vertical and horizontal furcation components rendering the effect of lycopene insignificant, which was in accordance with other studies.[28,29]

Only three studies have assayed 8-OHdG levels directly from the GCF.[11,12,30] In this study, all groups showed a 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 all previous studies in which periodontal treatment with or without lycopene therapy has been associated with a decrease in 8-OHdG levels. This could be due to the effect of scaling and root planning, which has been shown to contribute to a reduction in oxidative stress, as was demonstrated in a study that evaluated an NHG with collagen membrane treated group in relation to a xenograft and membrane group in periodontal regeneration.[21,28,31] Our results are also in accordance with a study that showed 2 mm of vertical furcation bone fill and 2 mm of vertical PD reduction after using anorganic bovine bone xenograft with a resorbable membrane, and yet another study that reported that nanocrystalline hydroxyapatite bone graft substitute in the treatment of intra-bony periodontal defects showed more reduction in PD when compared with the control group.[32,33]

To summarize, as the addition of lycopene to NHG covered with a resorbable collagen membrane did not significantly improve clinical parameters compared to the placebo group, we concluded that lycopene does not confer a benefit when combined with NHG in the surgical treatment of grade II furcation defects.

Acknowledgements: The authors would like to acknowledge and thank Dr. Shereen Sameh (Lecturer, Faculty of Pharmacy, October 6 University, Giza, Egypt) for formulation of the lycopene gel.

References

1. Tawfik MS, Abdel-Ghaffar KA, Gamal AY, El-Demerdash, FH, Gad HA. Lycopene solid lipid microparticles with enhanced effect on gingival crevicular fluid protein carbonyl as a biomarker of oxidative stress in patients with chronic periodontitis. *J Liposome Res*. 2019 Dec; 29(4): 375-382. https://doi.org/10.1080/08982104.2019.1566243

2. Meyle, J, Chapple I. Molecular aspects of the pathogenesis of periodontitis. *Periodontol 2000*. 2015 Oct; 69(1):7-17. https://doi.org/10.1111/prd.12104

3. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007; 39(1):44-84. https://doi.org/10.1016/j.biocel.2006.07.001

4. Masi S, Salpea KD, Li K, et al. Oxidative stress, chronic inflammation, and telomere length in patients with periodontitis. *Free Radic Biol Med*. 2011 Mar 15; 50(6):730-735. https://doi.org/10.1016/j.freeradbiomed.2010.12.031

5. Chiu BM, Zee KY, Corbet EF, Holmgren CJ. Periodontal implications of furcation entrance dimensions in Chinese first permanent molars. *J Periodontol*. 1991 May; 62(5):308-311. https://doi.org/10.1902/jop.1991.62.5.308

6. Reddy MS, Aichelmann-Reidy ME, Avila-Ortiz G, et al. Periodontal regeneration – furcation defects: a consensus report from the AAP Regeneration Workshop. *J Periodontol*. 2015 Feb; 86(2 Suppl):S131-133. https://doi.org/10.1902/jop.2015.140379

7. Canakci CF, Cicek Y, Yildirim A, Sezer U, Canakci V. Increased levels of 8-hydroxydeoxyguanosine and malondialdehyde and its relationship with antioxidant enzymes in saliva of periodontitis patients. *Eur J Dent*. 2009 Apr; 3(2):100-106.

8. Konopka T, Król K, Kopeć W, Gerber H. Total antioxidant status and 8-hydroxy-

2'-deoxyguanosine levels in gingival and peripheral blood of periodontitis patients. *Arch Immunol Ther Exp (Warsz)*. 2007 Nov-Dec; 55(6):417-422. https://doi.org/10.1007/s00005-007-0047-1

9. Di Mascio P, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys*. 1989 Nov 1; 274(2):532-538. https://doi.org/10.1016/0003-9861(89)90467-0

10. Chandra RV, Prabhuji ML, Roopa DA, Ravirajan S, Kishore HC. Efficacy of lycopene in the treatment of gingivitis: a randomized, placebo-controlled clinical trial. *Oral Health Prev Dent*. 2007; 5(4):327-36.

11. 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 May; 43(5):401-411.

12. 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 Jun; 43(3):121-129. https://doi.org/10.5051/jpis.2013.43.3.121

13. Michael G. *Burket's Oral Medicine, Twelfth Edition*. USA: PMPH-USA; 2015.

14. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Periodontol*. 2018 Jun; 89 Supp 1:S159-S172. https://doi.org/10.1002/JPER.18-0006

15. Gamal RM, Lotfy M, Taalab M. Evaluation of the efficacy of amniotic chorion membrane in management of grade II furcation defects (clinical study). *Alex Dent J*. 2018; 43(3):102-108. https://doi.org/10.21608/ADJALEXU.2018.58007

16. Pajnigara N, Kolte A, Kolte R, Pajnigara N, Lathiya V. Diagnostic accuracy of cone-beam computed tomography in identification and postoperative evaluation of furcation defects. *J Indian Soc Periodontol*. 2016; 20(4):386-390. https://doi.org/10.4103/0972-124X.192307

17. Silness J, Loe H. Periodontal Disease in Pregnancy. II. Correlation Between Oral Hygiene and Periodontal Condition. *Acta Odontol Scand*. 1964 Feb; 22:121-35. https://doi.org/10.3109/00016356408993968

18. Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L. A modified gingival index for use in clinical trials. *Clin Prev Dent*. 1986 Jan-Feb; 8(1):3-6.

19. Jain D, Sawhney A, Gupta B, et al. Clinical and Radiologic Evaluation of Regenerative Potential of NHA Paste and DBM in the Treatment of Periodontal Intra-bony Defects – A Randomized Control Trial. *J Clin Diagn Res*. 2016 Sep; 10(9):ZC74-ZC79. https://doi.org/10.7860/JCDR/2016/18268.8557

20. 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 Aug; 83(8):1018-1027. https://doi.org/10.1902/jop.2011.110281

21. Takane M, Sugano N, Iwasaki H, Iwano Y, Shimizu N, Ito K. New biomarker evidence of oxidative DNA damage in whole saliva from clinically healthy and periodontally diseased individuals. *J Periodontol*. 2002 May; 73(5):551-554. https://doi.org/1902/jop.2002.73.5.551

22. Takane M, Sugano N, Ezawa T, Uchiyama T, Ito K. A marker of oxidative stress in saliva: association with periodontally-involved teeth of a hopeless prognosis. *J Oral Sci*. 2005 Mar; 47(1):53-57. https://doi.org/10.2334/josnusd.47.53

23. Ali A, Saliem S, Abdulkareem A, Radhi H, Gul S. Evaluation of the efficacy of lycopene gel compared with minocycline hydrochloride microspheres as an adjunct to nonsurgical periodontal treatment: A randomised clinical trial. *J Dent Sci*. 2021 Mar; 16(2):691-699. https://doi.org/10.1016/j.jds.2020.09.009

24. Huang J, Best SM, Bonfield W, et al. In vitro assessment of the biological response to nano-sized hydroxyapatite. *J Mater Sci Mater Med*. 2004 Apr; 15(4):441-445. https://doi.org/10.1023/b:jmsm.0000021117.67205.cf

25. Herrera D. Scaling and Root Planning is Recommended in the Nonsurgical Treatment of Chronic Periodontitis. *J Evid Based Dent Pract*. 2016 Mar; 16(1):56-58. https://doi.org/10.1016/j.jebdp.2016.01.005

26. Chapple IL, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontol 2000*. 2007; 43:160-232. https://doi.org/10.1111/j.1600-0757.2006.00178.x

27. Arora N, Avula H, Avula JK. The adjunctive use of systemic antioxidant therapy (lycopene) in nonsurgical treatment of chronic periodontitis: a short-term evaluation. *Quintessence Int*. 2013; 44(6):395-405. https://doi.org/10.3290/j.qi.a29188

28. El Aawar N, Salah El-din M, Elfirt E, Shoeib M. Evaluating Nano-Hydroxyapatite in Periodontal Regeneration Using Cone Beam Volumetric Tomography. 2013.

29. Khanna D, Malhotra S, Naidu DV. Treatment of grade II furcation involvement using resorbable guided tissue regeneration membrane: A six-month study. *J Indian Soc Periodontol*. 2012 Jul; 16(3):404-410. https://doi.org/10.4103/0972-124X.100920

30. Dede FÖ, Ozden FO, Avcı B. 8-hydroxy-deoxyguanosine levels in gingival crevicular fluid and saliva in patients with chronic periodontitis after initial periodontal treatment. *J Periodontol*. 2013 Jun; 84(6):821-828. https://doi.org/10.1902/jop.2012.120195

31. Ekuni D, Tomofuji T, Tamaki N, et al. Mechanical stimulation of gingiva reduces plasma 8-OHdG level in rat periodontitis. *Arch Oral Biol*. 2008 Apr; 53(4):324-329. https://doi.org/10.1016/j.archoralbio.2007.10.005

32. Houser BE, Mellonig JT, Brunsvold MA, Cochran DL, Meffert RM, Alder ME. Clinical evaluation of anorganic bovine bone xenograft with a bioabsorbable collagen barrier in the treatment of molar furcation defects. *Int J Periodontics Restorative Dent*. 2001 Apr; 21(2):161-169.

33. Heinz B, Kasaj A, Teich M, Jepsen S. Clinical effects of nanocrystalline hydroxyapatite paste in the treatment of intrabony periodontal defects: a randomized controlled clinical study. *Clin Oral Investig*. 2010 Oct; 14(5):525-531. https://doi.org/10.1007/s00784-009-0325-x

Conflicts of interest: The authors declared no conflicts of interest related to this work.

Corresponding author:

Dr. Dalia Shawky Gaber

Assistant Lecturer, Department of Oral Medicine, Periodontology, Oral Diagnosis, and Oral Radiology
Faculty of Dental Medicine
October 6 University
Giza, Egypt

E-mail: dahliashawky86@hotmail.com
Phone: +20 100 6196397