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# The effects of subgingival application of ozonated olive oil gel in Smokers with periodontitis (Clinical and Bacteriological study)

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***Abstract:***
***Background****: smoking is widely regarded the strongest modifiable risk factor for periodontal disease progression and has been associated with increased risk of periodontal attachment loss and poorer clinical outcome of non-surgical or surgical periodontal therapy, so adjunctive therapies may prove to be more effective than conventional treatment only. This study evaluates the effect of the subgingival application of ozonated olive oil gel as an adjunct to scaling and root planning (SRP) in smokers with periodontitis.*
***Materials and Methods****: Thirty participants were involved in this study.15 subjects were non smokers and 15 were smokers. Both groups were diagnosed with stage II periodontitis. A split-mouth randomized clinical study was conducted in all 30 patients receiving scaling and root planning alone in randomly selected quadrants and scaling and root planning with the subgingival application of ozonated olive oil gel in the other quadrants. Periodontal Clinical parameters, plaque sample were performed at baseline and after 6 weeks for all patients. Plaque Samples were cultured anaerobically for detection of p.gingivalis and A.actinomycetemcomitans.*
***Results****: regarding bacterial count, there was a higher significant reduction in scaling and root planning combined with ozonated olive oil gel compared to the results of scaling and root planning alone. There was more favorable outcome in all clinical parameters in scaling and root planning combined with ozonated olive oil gel compared to SRP only but the difference was statistically non-significant*
***Conclusion:*** *Adjunctive application of ozonated olive oil gel in smokers with periodontitis may provide additional benefit as an adjunct to SRP.*
***Key Word****: Periodontitis; Smoking; Ozone.*

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## I. Introduction

periodontal diseases involve an interaction between various factors as the subgingival microbiota, the host immune and inflammatory responses, and environmental factors that can modify the host response , so it cannot be considered simple bacterial infections. Rather than that, it is are multifactorial complicated disease.(1) Modifying factors are defined as any agent or condition that changes an individual's response to subgingival plaque accumulation (e.g., smoking, systemic conditions, medications).

Smoking is the single most powerful avoidable or modifiable risk factor for periodontitis, increasing the risk by 85 percent. (2) A dose-response connection exists between the number of cigarettes smoked per day and the likelihood of developing periodontitis, with ORP = 2.79 for 9 cigarettes per day and ORP = 5.88 for 31 cigarettes per day. .(3)

adjunctive therapies have been advocated for smokers to enhance the outcome of mechanical debridement, these include systemic and local antimicrobials(4)

One of these antimicrobials is ozone. It is a naturally occurring gas and a powerful oxidizing agent so it is used in treatment of various disease including periodontal diseases.

## II. Material And Methods

This study was conducted after approval by the Ethics Committee, Faculty of Dentistry, Mansoura University,Egypt. It included 30 apparently healthy individuals (15 smokers and 15 non-smokers) that were selected from patients attending the outpatient clinic Department of Periodontology and Oral Medicine, Faculty of Dentistry, Mansoura University.

**Study Design:** Split mouth prospective study
**Study Location**: Outpatient clinic Department of Periodontology and Oral Medicine, Faculty of Dentistry

**Study Duration:** six weeks
**Sample size:** 30 patients.
**Subjects & selection method**:
**The patients were divided into 2 groups as follow:**
**Group (I) (smokers): n=15** subdivided into:
**-Test side** treated with scaling& root planing and adjunctive use of ozonated olive oil gel as local antimicrobial drug.
**- Control side** treated with scaling and root planing only
**Group (II) (non smokers) : n=15** subdivided into:
**- Test side** treated with scaling& root planning and adjunctive useof ozonated olive oil gel as local antimicrobial drug.
**- Control side** treated with scaling and root planning only.

**Inclusion criteria for smokers group:**
1. Patient with stage II periodontitis
2. Either sex
3. Aged 25 to 40
4. Smoke more than 10 cigarettes per day for at least 5 years.

**Inclusion criteria for non-smokers group:** same as smokers group except smoking

**Exclusion criteria:**
1. Pregnant and lactating women;
2. Patients with systemic disease, such as diabetes or a blood disorder
3. Patients who have had antibiotics, nonsteroidal anti-inflammatory medications, or corticosteroids in the preceding six month.
4. Patients who had received periodontal treatment throughout the past six months
5. Immunocompromised patients
6. Patients with glucose-6-phosphate-dehydrogenase deficiency

**Procedure methodology**

At the initial appointment, all patients in both groups had their plaque and gingival indices examined and documented.

Each quadrant is scaled and root planned using an ultrasonic scaler and manual instruments until tactile sensation detects smooth crown and root surfaces. Local anaesthetic was utilized in some instances where it was necessary. Oral hygiene guidelines have been reinforced at each consultation.

A disposable plastic syringe was used to inject ozonated olive oil gel subgingivally into selected quadrant deepest periodontal pocket.

Gel application was carried out following the first SRP and at 2, 4 weeks. Oral hygiene advices have been given at each consultation.

Subgingival plaque samples were taken from all individuals in all groups before to and after six weeks of therapy.

The samples are inserted in 1.5 ml of thyoglycolate broth transport media and sent immediately to department of microbiology and immunology for sample processing. three bacterial media were used, first one is brain heart infusion agar for bacterial total count detection, second one is brain heart infusion agar mixed with 5.0 ug/ml hemin for detection of P. gingivalis and third medium contains tryptic soy agar (40 g/L), yeast extract (1.0 g/L), 10% horse serum, Bacitracin (75 ug/mL), and Vancomycin (5 ug/mL). for the isolation of Aggregatibacter actinomycetemcomitans

Ozonated olive oil is prepared by bubbling of ozone-oxygen mixture until olive oil transforms from the greenish-colored liquid to the whitish gel status.

**Statistical analysis**

Data were analyzed using SPSS program for Windows (version 22).
Student t test was used to compare smokers and non-smokers groups.Paired t test was used to compare baseline

and after 6 weeks.
Spearman's correlation was used determine the correlation between clinical indices and bacterial count..
Z test was used for comparing percent of change between 2 groups

## III. Result

At baseline there was no significant differences between groups regarding age and sex.

**Non-smokers group:**
**At baseline**, non-smokers group showed non statistically significant difference between test and control sides regarding clinical indices and bacterial count.
**After treatment** there was a statistically significant decrease of all clinical indices after 6 weeks for both sides compared to baseline but the difference between both sides after treatment was non statistically significant.

**Regarding bacterial count,**
- **At baseline** there was no statistically significant difference between test side and control side.

**- After 6 weeks** , there was a high statistically significant difference between both test and control sides of non smokers group regarding total bacterial count (269.33) versus (421.33) and also P.gingivalis count ( 6.93±5.11) versus (11.27±3.34) and A. actinomycetemcomitans count (3.0±1.25) versus (5.2±1.89) with P values (P=0.001), (P=0.01) and (P = 0.01) respectively.(Table 1 )

**Table no 1 :** comparison of bacterial count between control and test side of the non-smokers group

| | | Non-smokers | | Test of significance |
|---|---|---|---|---|
| | | Control side n=15 | test side n=15 | |
| **Total bacterial count ($\times 10^3$)** | Baseline | 906.66±82.15 | 885±75.40 | t=0 p=1.0 |
| | After 6 weeks | 421.33±53.03 | 269.33±57.59 | t=7.52 p=0.001* |
| **Paired t test** | | P<0.001* | P<0.001* | |
| **% of change** | | 53.5% | 69.6% | p=0.365 |
| **A.actinomycetemcomitans($\times 10^3$)** | Baseline | 12.67±4.05 | 12.80±5.06 | t=0.08 p=0.937 |
| | After 6 weeks | 5.20±1.89 | 3.0±1.25 | t=7.62 p=0.01* |
| **Paired t test** | | P<0.001* | P<0.001* | |
| **% of change** | | 58.9% | 77.4% | p=0.277 |
| **P. gingivalis ($\times 10^3$)** | Baseline | 25.60±4.97 | 26.40±6.33 | t=0.385 p=0.703 |
| | After 6 weeks | 11.27±3.34 | 6.93±5.11 | t=2.74 p=0.01* |
| **Paired t test** | | P<0.001* | P<0.001* | |
| **% of change** | | 55.9% | 73.7% | P=0.307 |

**Smokers group**
**At baseline**, smokers group showed non statistically significant difference between test and control sides regarding clinical indices and bacterial count
**After treatment** there was a statistically significant decrease of all clinical indices after 6 weeks for both sides compared to baseline but the difference between both sides after treatment was non statistically significant

**Regarding bacterial count**,
- **At baseline** there was no statistically significant difference between test side and control side.
- **After 6 weeks**, there is a high statistically significant difference between test and control sides regarding total bacterial count (488±54.79) versus (576.67±67.47), P.gingivalis count(12.80±5.49) versus (18.40±6.19) and A. actinomycetemcomitans count (9.0±1.45) versus (10.33±1.76) with P values (P=0.001), (P=0.014) and (P=0.03) respectively .(Table 2)
**In comparison**, there was a non-statistically significant difference between percent of change in clinical indices and bacterial count between smokers and non-smokers for both sides after 6 weeks, however the percent of change was always higher in non smokers group except for gingival index.

**Table no 2 :** comparison of bacterial count between control and test side of the studied smokers group

| | | Smokers | | test of significance |
|---|---|---|---|---|
| | | Control side n=15 | test side n=15 | |
| **Total bacterial count($\times10^3$)** | Baseline | 1062±105.30 | 1042±98.72 | t=0.537 p=0.596 |
| | After 6 weeks | 576.67±67.47 | 488±54.79 | t=3.95 p=0.001* |
| **Paired t test** | | P<0.001* | P<0.001* | |
| **% of change** | | 45.6% | 53.2% | p=0.677 |
| **A.actinomycetemcomitans($\times10^3$)** | Baseline | 19.07±2.91 | 18.80±3.61 | t=0.223 p=0.825 |
| | After 6 weeks | 10.33±1.76 | 9.0±1.45 | t=1.82 p=0.03* |
| **Paired t test** | | P<0.001* | P<0.001* | |
| **% of change** | | 45.8% | 52.1% | p=0.730 |
| **P.gingivalis($\times10^3$)** | Baseline | 31.87±5.88 | 32.67±5.49 | t=0.385 p=0.727 |
| | After 6 weeks | 18.40±6.19 | 12.80±5.49 | t=2.62 p=0.014* |
| **Paired t test** | | P<0.001* | P<0.001* | |
| **% of change** | | 42.3% | 60.8% | p=0.311 |

## IV. Discussion

Dyslipidemia Several studies have revealed that smoking might have an impact on the subgingival microbiome. Smokers had a greater incidence of periodontal infections such as Porphyromonas gingivalis, Tannerella forsythia, and Aggregatibacter actinomycetemcomitans than nonsmokers(5,6).

Chronic tobacco use lowers perfusion as a result of repeated vasoconstrictive shocks and microvasculature remodelling. In periodontal disease, microbe-mediated tissue damage results in the upregulation of endothelial adhesion molecules, which promotes leucocyte attraction, resulting in persistent inflammation and angiogenesis. These mechanisms are inhibited in chronic tobacco smokers, since proinflammatory cytokines and proangiogenic factors are lowered (7).

A recent systematic review by Chambrone et al (4) found that using local delivery antibiotics as an adjunct to non-surgical periodontal therapy improved clinical periodontal parameters in smokers when compared to non-surgical therapy alone. However, adjunctive use of systemic antibiotics did not improve clinical periodontal parameters when compared to non-surgical therapy alone

Periodontal pockets in smokers have a very low oxygen tension(8), which might provide an ideal habitat for anaerobic periodontal bacteria to grow, even in shallow pockets. This was supported Eggert et al.(9) clinical findings that smoking even in at shallow sites (≤5 mm) creates a favorable environment for bacteria, such as P. gingivalis, Prevotella intermedia, and A. actinomycetemcomitans.

In this context, ozone therapy was considered to be used in this study as an adjunctive to scaling and root planning as a possible alternative local antimicrobial agent with anti-hypoxic effect(10,11). Ozone has been shown to be biocompatible with oral epithelial cells, periodontal cells, and gingival fibroblasts by Azarpazhooh et al (12).

The study used ozonated olive oil gel over ozonated water because gels were proven to have a prolonged stay in the oral cavity, appropriate drug penetration, high effectiveness, and tolerability (13)

The clinical parameters (GI, PPD, CAL, PI ) were recorded at baseline and after 6 weeks. Cugini et al (14) showed that the biggest reduction in probing depth and increase in clinical attachment occurred 1–3 months after scaling..

At baseline, both groups had no significant difference between test and control sides.

A significant reduction in mean values for clinical parameters (PI, GI, PPD, and CAL) has been achieved in this study in both sides (test and control side) in both groups ( smokers and non smokers ), after 6 weeks as compared to baseline status.

But there was non significant difference between both sides after treatment in both groups.

This non significant difference between two sides after treatment can be explained by the significant effect of scaling and root planning in removing plaque and calculus in both groups, decreasing the microbial biofilm which in turn result in lower levels of inflammation and better clinical parameters.(15)

The findings agree with Al Habashneh et al(16) who found that irrigation with ozonated water as an adjuvant

therapy to SRP had no statistically significant effect when compared to SRP plus distilled water irrigation.
Also our findings agree with Moraschini et al(17), who demonstrated that while current evidence indicates that ozone has good antimicrobial activity and is biocompatible with periodontal and gingival cells, no evidence was found to support its use as a scaling and root planing adjunct. This review focused exclusively on ozone in its aqueous or gaseous state.
Thome et al in another more recent systematic review(18) included studies with all forms of ozone ( gas, water or oil ) and also showed that provides very limited additional benefits in terms of PDD reduction and CAL gain.
The exception of gingival index may be attributed to the well established effect of tobacco smoking on gingival tissue bleeding which had been confirmed by Buduneli et al and Peruzzoet al that tobacco smoking suppress the vascular response of gingiva.(19,20)

While the findings differ from those of Patel et al(21) and Shoukheba et al(22), according to their results there is a significant improvement of clinical parameters for test group ( SRP plus ozonated olive oil ) in comparison to the control groups ( SRP only ) over time. This might be explained by our study's short follow-up time (6 weeks ) while their studies followed the patients 2-6 months.
Regarding the microbiological evaluation of total bacterial count, P.gingivilis and A.actinomycetemcomitans , the present study revealed non significant difference, between test and control sides of each group at baseline, however the mean bacterial count was higher in smokers group at baseline.
After 6 weeks, there was a statistically significant reduction of total bacterial count, P.gingivilis and A.actinomycetemcomitans for both control and test sides of both groups compared to baseline.
There was also a greater reduction in bacterial count in test side compared to control sides of both groups, this was statistically significant for total bacterial count, P.gingivilis and A.actinomycetemcomitans.

These finding agree with Gandhi et al(23), as they demonstrated significant intragroup reduction in P. gingivalis and A.actinomycetemcomitans count from baseline to 3 months follow-up. Also it agree with Patel et al(21) and lendhey et al(24),as they demonstreated significant reduction in bacterial colonies observed after anaerobic incubation in the ozone oil group at baseline and 1 month.

Our results disagree with Kshitish and Laxman et al.(25) who studied the effect of ozone on P. gingivalis and T. forsythia and found no significant antibacterial effect. The author addressed the likely reason for this discrepancy in results by stating that the PCR method may detect both living and non-viable bacteria. Thus, if non-viable microorganisms are identified during antimicrobial therapy, determining the antimicrobial agent's efficiency becomes challenging, whereas the culture method utilized in this study detects only live bacteria.
The percentage of improvement in all clinical parameters and bacterial count was always higher in non smoker group than smoker group in test or control side with exception of ginigival index .
This is in agreement with several studies which have been recently reviewed by Chang et al(26),who demonstrated that smoking had a deleterious influence on clinical responses to non-surgical periodontal treatment, with smokers having considerably less PD reduction and CAL increase than non-smokers

## V. Conclusion

1. Scaling and root planning remain the most important key to successful periodontal therapy in patient with moderate periodontitis.
2. Smoking has a negative effect on severity of periodontal disease and response to treatment.
3. Adjunctive subgingival application of ozonated olive oil represent a natural moderate antiseptic that may provide additional benefit to SRP and more improvement in clinical parameters.

## References

[1]. Bartold PM, Van Dyke TE. Periodontitis: a host-mediated disruption of microbial homeostasis. Unlearning learned concepts. Periodontol 2000. 2013 Jun 25;62(1):203–17.
[2]. Leite FRM, Nascimento GG, Scheutz F, López R. Effect of Smoking on Periodontitis: A Systematic Review and Meta-regression. Am J Prev Med. 2018;54(6):831–41.
[3]. Tomar SL, Asma S. Smoking-Attributable Periodontitis in the United States: Findings From NHANES III. J Periodontol. 2000;71(5):743–51.
[4]. Chambrone L, Vargas M, Arboleda S, Serna M, Guerrero M, de Sousa J, et al. Efficacy of Local and Systemic Antimicrobials in the Non-Surgical Treatment of Smokers With Chronic Periodontitis: A Systematic Review. J Periodontol. 2016;87(11):1320–32.
[5]. Huang C, Shi G. Smoking and microbiome in oral, airway, gut and some systemic diseases. J Transl Med. 2019;17(1):1–15.
[6]. Guglielmetti MR, Rosa EF, Lourenção DS, Inoue G, Gomes EF, De Micheli G, et al. Detection and Quantification of Periodontal Pathogens in Smokers and Never-Smokers With Chronic Periodontitis by Real-Time Polymerase Chain Reaction. J Periodontol. 2014;85(10):1450–7.
[7]. Silva H. Tobacco use and periodontal disease—the role of microvascular dysfunction. Biology (Basel). 2021;10(5):1–27.
[8]. Hanioka T, Tanaka M, Takaya K, Matsumori Y, Shizukuishi S. Pocket Oxygen Tension in Smokers and Non-Smokers With Periodontal Disease. J Periodontol. 2000;71(4):550–4.

[9]. Eggert F-M, McLeod MH, Flowerdew G. Effects of Smoking and Treatment Status on Periodontal Bacteria: Evidence That Smoking Influences Control of Periodontal Bacteria at the Mucosal Surface of the Gingival Crevice. J Periodontol. 2001 Sep 1;72(9):1210–20.
[10]. Saini R. Ozone therapy in dentistry: A strategic review. J Nat Sci Biol Med. 2011;2(2):151–3.
[11]. Vasthavi C, Babu H, Rangaraju V, Dasappa S, Jagadish L, Shivamurthy R. Evaluation of ozone as an adjunct to scaling and root planing in the treatment of chronic periodontitis: A randomized clinico-microbial study. J Indian Soc Periodontol. 2020;24(1):42.
[12]. Azarpazhooh A, Limeback H. The application of ozone in dentistry: A systematic review of literature. J Dent. 2008;36(2):104–16.
[13]. Ugazio E, Tullio V, Binello A, Tagliapietra S, Dosio F. Ozonated oils as antimicrobial systems in topical applications. Their characterization, current applications, and advances in improved delivery techniques. Molecules. 2020;25(2):1–24.
[14]. Cugini MA, Haffajee AD, Smith C, Kent RL, Socransky SS. The effect of scaling and root planing on the clinical and microbiological parameters of periodontal diseases: 12-month results. J Clin Periodontol. 2000;27(1):30–6.
[15]. Cobb CM. Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. J Clin Periodontol. 2002 May;29:22–32.
[16]. Al Habashneh R, Alsalman W, Khader Y. Ozone as an adjunct to conventional nonsurgical therapy in chronic periodontitis: A randomized controlled clinical trial. J Periodontal Res. 2015;50(1):37–43.
[17]. Moraschini V, Kischinhevsky ICC, Calasans-Maia MD, Shibli JA, Sartoretto SC, Figueredo CM, et al. Ineffectiveness of ozone therapy in nonsurgical periodontal treatment: a systematic review and metaanalysis of randomized clinical trials. Clin Oral Investig. 2020;24(6):1877–88.
[18]. Thomé DP, Wilker F, Gomes M, Nicolini AC, Kuchenbecker C. The Effect of Adjuvant Ozone Therapy in Nonsurgical Periodontal Treatment : A Systematic Review with Meta-analysis. 2021;266–81.
[19]. Buduneli N, Scott DA. Tobacco-induced suppression of the vascular response to dental plaque. Mol Oral Microbiol. 2018;33(4):271–82.
[20]. Peruzzo DC, Gimenes JH, Taiete T, Casarin RCV, Feres M, Sallum EA, et al. Impact of smoking on experimental gingivitis. A clinical, microbiological and immunological prospective study. J Periodontal Res. 2016;51(6):800–11.
[21]. Patel P V., Patel A, Kumar S, Holmes JC. Effect of subgingival application of topical ozonated olive oil in the treatment of chronic periodontitis: a randomized, controlled, double blind, clinical and microbiological study. Minerva Stomatol. 2012;61(9):381–98.
[22]. Shoukheba MYM, Ali SA. The effects of subgingival application of ozonated olive oil gel in patient with localized aggressive periodontitis. A clinical and bacteriological study. Tanta Dent J. 2014;11(1):63–73.
[23]. Gandhi KK, Cappetta EG, Pavaskar R. Effectiveness of the adjunctive use of ozone and chlorhexidine in patients with chronic periodontitis. BDJ Open. 2019;5(1):3–6.
[24]. Lendhey S, Kale T, Seth T, Bhartiya G, Hudwekar A. Effect of subgingival application of ozone oil versus olive oil as an adjunct to scaling and root planing in chronic periodontitis: A clinical and microbiological study. J Oral Res Rev. 2020;12(2):63.
[25]. Kshitish D, Laxman VK. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: A clinical and microbiologic study. Indian J Dent Res. 2010;21(3):341–8.
[26]. Jiang Y, Zhou X, Cheng L, Li M. The Impact of Smoking on Subgingival Microflora: From Periodontal Health to Disease. Front Microbiol. 2020;11(January):1–13.

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