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Review Article

Clinical and Microbiological Evaluation of Oleozone Gel in the Treatment of Chronic Periodontitis

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Abstract

Conventional non-surgical periodontal therapy has been proven to be an effective treatment for patients with chronic periodontitis. Local administration of antimicrobials provides a high concentration of the drug in the pocket and enhances the clinical results without any systemic side effects and bacterial resistance. However, a new trend is to diminish the use of antibiotics to avoid resistant strains. Sixteen patients were divided into two groups. Group I (control group), group II : and group II : SRP + oleozone gel. Clinical parameters and *P. gingivalis* count via PCR were recorded at 1, 3 and 6 months. Inter-group comparison revealed that group II showed more favorable results both clinically and microbiologically. Hence, adjunctive application of oleozone gel represents a promising natural product as adjunct to SRP.

Keywords: *Oleozone; Chronic Periodontitis; P. gingivalis; PCR Test; Full Mouth Scaling and Root Planning (FM-RP)*

Abbreviations

P. gingivalis: *Porphyromonas gingivalis*; ROS: Reactive Oxygen Species; PG-E2: Prostaglandin E2; SRP: Scaling and Root Planning; PMNS: Polymorphonuclear Neutrophils; IL: Interleukin; PD: Pocket Depth; CAL: Clinical Attachment Level; HBO: Hyperbaric Oxygenation; HBOT: Hyperbaric Oxygen Therapy; BOP: Bleeding on Probing; PGE2: Prostaglandin E2; UNC: University of North California; GCF: Gingival Crevicular Fluid

Introduction

Periodontitis is a chronic infection involving destruction of the tooth-supporting apparatus, including the periodontal ligament and alveolar socket support of the teeth. Periodontal disease is initiated by a local accumulation of bacteria and their metabolic products that leads to apical migration of the junctional epithelium along the root surface, deepening the gingival crevice to produce periodontal pockets and associated attachment loss, which is the hallmark lesion of periodontal disease [1].

The microbiology of periodontal infections is quite complicated, and numerous bacterial agents have been implicated in their etiology [2,3]. *P. gingivalis* has been implicated as a major etiologic agent in the development of adult periodontitis. *P. gingivalis is* a highly adapted pathogen that is armed with a number of putative virulence factors that enable this organism to cause disease. Among such putative virulence factors are fimbriae and lectin-type adhesins, a polysaccharide capsule, lipopolysaccharide, hemagglutination and hemolysing activities, toxic products of metabolism, outer membrane vesicles, and numerous enzymes [4].

Moreover, neutrophils play a pivotal role in host defense and are the first line of defense against this infectious periodontal disease. Neutrophils have several selective mechanisms for controlling bacterial invasion, including both intracellular and extracellular oxidative and non-oxidative killing mechanisms. The oxidative killing mechanism of neutrophils and other phagocytes involves the formation of reactive oxygen species (ROS) [5,6].

While most ROS have extremely short half-lives, they can cause substantial tissue damage through ground substance degradation, collagenolysis either directly or indirectly or as a result of oxidation of proteases, stimulation of excessive pro-inflammatory cytokine release, prostaglandin E2 (pG-E2) production via lipid peroxidation and superoxide release, both of which have been linked with periodontal inflammation, subsequent attachment loss and bone resorption [6].

Therefore, primary objectives of therapy for patients with chronic periodontitis are to halt disease progression and to resolve inflammation. Therapy at a diseased site is aimed at reducing etiologic factors below the threshold capable of producing breakdown, allowing repair of the affected region. Regeneration of lost periodontal structures can be enhanced by specific procedures either through non-surgical periodontal therapy (scaling and root planning (SRP) and the adjunctive use of chemotherapeutic agent) or surgical therapy [7].

Unfortunately, in some instances, the complex anatomy of the root and the contours of the lesion may hamper the treatment and prevent sufficient reduction of the bacterial load to make the tooth surface biologically acceptable [8].

This could be related to the persistence of pathogens in the pocket after treatment or to the production of specific virulence factors by the bacteria interfering with the host defense. In this context, it is evident that antimicrobial agents are of great interest and may be valuable as adjuncts to mechanical therapy. These adjunctive therapies are categorized by their route of administration to systemic or local drug delivery [7].

Several locally delivered antimicrobials have been used for periodontal therapy as tetracycline, metronidazole gel, minocycline, doxycycline, and azithromycin, etc [9,10].

It is worthy noted that there is still a possibility of resistant bacterial strains following local delivery, which seem to disappear after 3 to 6 months. There are no definitive data on the possible adverse effects of subgingival antibiotic slow-release devices on the microbiota of the gastrointestinal tract. This lack of data has spurred speculations and concerns of possible spread of bacterial resistance and even increases in the likelihood of the transfer of multi-drug resistance after local application of antibiotics [11].

Therefore, a new concept is directed toward preventing the disease not only by inhibiting the putative pathogens but also by interfering with the factors responsible for the transition of the plaque microflora from the commensal to a pathogenic relationship with the host [12].

One of the alternative approaches to conventional antimicrobial agents in the suppression of subgingival bacteria is to inhibit their growth by changing the subgingival environment, which has been shown to be highly anaerobic with a prevailing low oxygen tension as advocated by Dunlop in 1913. Various agents such as molecular oxygen, hyperbaric oxygenation (HBO) [13], hydrogen peroxide [14] and ozone therapy have been applied [12]. Various agents have been applied, such as molecular oxygen, hyperbaric oxygenation, hydrogen peroxide and ozone products [15].

Ozone has been shown to possess unique properties and has potential applications to the clinical practice of dentistry. There are several known actions of ozone such as antimicrobial, immunostimulating, acceleration of the wound healing rate. Additionally, it is anti-hypoxic through rising of oxygen tension (pO_2) in tissues and improves transportation of oxygen in blood. Moreover, it is a powerful antioxidant and radical scavenger, where direct contact of Ozone gel with host cells leads to decrease release of reactive oxygen species [16].

The aim of this study will be to assess the clinical, microbiological, and growth factor activity of ozone therapy versus 0.5% azithromycin gel on the treatment of moderate chronic periodontitis.

Materials and Method

Participants enrolled in this study presented to Tanta University, faculty of Dentistry with moderate chronic periodontitis, CAL measuring 3 - 4 mm. Only single rooted teeth were included. Approval for this project was obtained from Tanta Faculty of Dentistry, Tanta University Research Ethics Committee (REC). All individuals completed a written and verbal consent to participate.

Enrolled volunteers met the following inclusion criteria: ≥ 35 years of age, able to understand and comply with all instructions, and able to maintain good oral hygiene and, systemically healthy patients. CAL measuring 3 - 4 mm with no gingival recession.

Exclusion criteria included history of antibiotic, anti-inflammatory drugs or periodontal therapy in the preceding 6 months. Patients with risk factors (e.g.- smoking unstable systemic diseases), known allergy to any materials used. In addition, women who were pregnant or attempting to become pregnant were excluded.

Materials

Oleozon gel (Figure 1)*: Oleozon is a pure olive oil that has undergone ozonization using a steady flow of ozone-oxygen mixture in the ratio of 5:95 % until olive oil transforms from the greenish-colored liquid status to the whitish gel status, Placebo gel (methyl cellulose). A customized made acrylic stent will be fabricated and a calibrated standard periodontal probe (UNC-15) will be used to standardize the measurements of the probing pocket depth (PPD) and clinical attachment level [CAL], Polymerase chain reaction kit for bacteriological examination** (Figure II).

Figure 1: *Ozonated olive oil gel.*

Figure 2: *PCR test kit for P. gingivalis.*

Patients will be randomly assigned into the following treatment protocols (using sealed envelopes):- Group I: - (Control group) received conventional periodontal treatment including full mouth scaling and root planing (SRP) + placebo gel), Group II: received (SRP) + subgingivally delivered ozonated olive oil gel.

Full mouth periodontal charting was done for all patients. This is followed by fabrication of acrylic occlusal stent for repeated positioning of the probe. All patients were scheduled for one session full mouth SRP using ultrasonic scalers and hand instrumentation and polishing.

Immediately, after completion of SRP the prepared gel will be injected into the deepest part of the periodontal pockets using a blunt canula until resistance is felt. No periodontal dressing will be applied after placement of the drug (Figure 3).

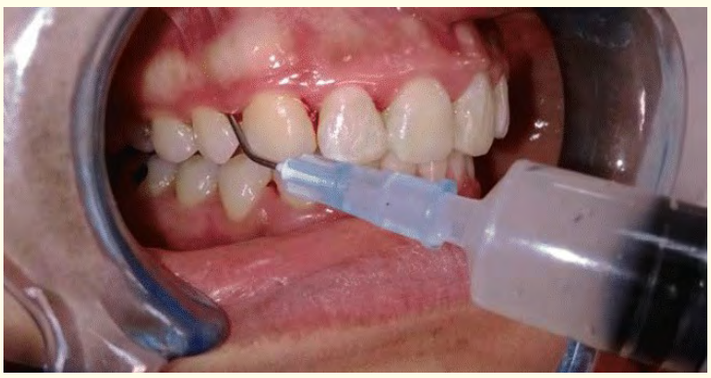

Figure 3: *Application of the gel by blunt needle immediately after SRP.*

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** primer design, UK

After placement of the in-situ gel, patients will be instructed to refrain from chewing hard or sticky food, brushing, or using any interdental aids. Instructions with supragingival brushing at the site of application will be given for at least 2 days after application. Adverse effects will be noted at subsequent appointments, and any supragingival deposits will be scaled. No antiplaque agents, systemic antibiotics or anti-inflammatory drugs will be prescribed or allowed during the study design. Reinforcement of oral hygiene instructions at each appointment.

The following clinical parameters recorded, at baseline (before treatment), one, three and six months following the treatment for all patients :Probing pocket depth (PPD), Clinical attachment level [CAL] [17], Bleeding on probing (BOP) and Plaque index (PI).

Plaque samples were collected for PCR analysis Each previously selected tooth was isolated with sterile cotton rolls, and the supragingival plaque was removed with sterile cotton pellets. A sterilized paper point was carefully inserted to the maximum depth of the periodontal pocket and held in position for 10 seconds. The paper point was then placed in 1 ml phosphate buffered saline (PBS) containing 0.1% silica particles (Figure 4).

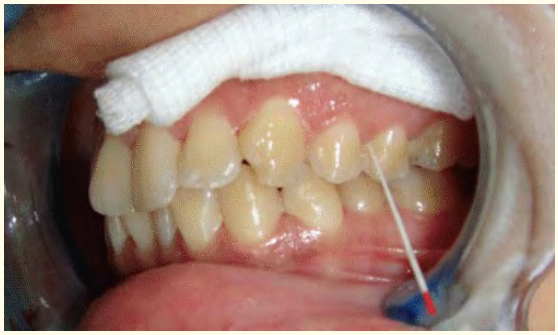

Figure 4: *Sample collection via sterile paper point.*

Data analysis

Each examined site will be considered as a unit of analysis. All the results were tabulated and statistically analyzed using Statistical package for social science (SPSS version 20). For evaluation of the significance of the effect of the various treatment modalities (oleozone gel -placebo gel) on the clinical parameters (probing-attachment level-BOP-PI), bacteriological parameters measured by RT-PCR, the following test were used: Paired student T test: for comparison of the studied parameters scores taken at baseline with those taken at subsequent evaluation periods (BL-1M-3MS 6MS) for all treatment modalities (intragroup comparison).

Results

All patients were followed up over the entire 6 months study period (one, 3, and 6 months) with no drop out. The performed three treatment modalities were well tolerated by all the patients without any complications or adverse effects.

The baseline measurements showed no statistically significant differences among the different treatment modalities for any of the measured clinical, bacteriological, and immunological parameters as evident by their mean baseline values ($P > 0.05$).

The intergroup comparison demonstrated that, group II showed a significant reduction in the mean values of BOP at 3 months (0.25 ± 0.46) ($P < 0.05$) when compared to group I (0.87 ± 0.35). Also, they showed a significant reduction in the mean values of PI at 6 months (0.87 ± 0.46) when compared to group I (2.12 ± 0.7).

On the other hand, group II showed significant reduction in PPD and CAL till 6 months (1.25 ± 0.35, 0.11 ± 0.33) as compared to group I (3.25 ± 0.88, 0.625 ± 0.5) (2.12 ± 0.83).

Bacteriological results showed that, Favorable scores were noticed with group II which recorded a highly significant continued reduction of the *P. gingivalis* count till 3 months with non-significant deterioration at 6 months. GII results showed a remarkable improvement of PCR results at 1 month (25.58 ± 5.33) which continued till 3 months (23.60 ± 3.74) as compared to their mean base line value (34.65 ± 1.07). However, a slight deterioration was recorded at 6 months (24.45 ± 3.48) but still below the baseline value. T test showed that there were a statistically highly significant reduction in the bacterial load by comparing 1,3 and 6 months to the base line value ($P < 0.001$). However, no statistical changes were recorded when comparing 1, 3, and 6 months to each other ($P > 0.05$).Comparing group I and group II showed that, the mean PCR reduction was statistically non- significant at 1 month ($P > 0.05$). While at 3 and 6 months a highly significant improvement was recorded in favor of group II ($P < 0.001$).

Clinical Parameters	Baseline	1 month	3 months	6 months
CAL Oleozone Control	 2.44 ± 0.5 2.37 ± 0.5	 0.44 ± 0.7 0.25 ± 0.4	 0.11 ± 0.33 0.50 ± 0.7	 0.11 ± 0.33 0.62 ± 0.5
P value		0.52	0.10	0.01*
PD Oleozone Control	 4.5 ± 0.5 4.37 ± 0.51	 2.37 ± 0.9 2.25 ± 0.46	 1.5 ± 0.5 2.5 ± 0.7	 1.25 ± 0.35 3.25 ± 0.88
P value		0.73	0.008**	0.0001**
BOP Oleozone Control	 1 ± 0.0 1 ± 0.0	 0.12 ± 0.35 0.37 ± 0.51	 0.25 ± 0.46 0.87 ± 0.35	 0.5 ± 0.53 0.87 ± 0.35
P value		0.35	0.01*	0.59
PI Oleozone Control	 1.71 ± 0.45 2.09 ± 0.8	 0.65 ± 0.53 1.31 ± 0.70	 0.46 ± 0.36 0.39 ± 0.74	 0.87 ± 0.46 2.12 ± 0.7
P value		0.05	0.29	0.0003*

Table 1: *Changes in clinical parameters between base line and 6months.*

Time of Assessment		GI	GII
Baseline (BL)		31.60 ± 5.706	34.65 ± 1.07
1 month (1 m)		28.49 ± 4.49	25.58 ± 5.33
3 months (3 ms)		30.99 ± 4.7	23.60 ± 3.74
6 months (6 ms)		36.21 ± 3.17	24.45 ± 3.48
T-test	BL and 1 m	0.003**	0.002**
	BL and 3 ms	0.22	0.0001**
	BL and 6 ms	0.002**	0.0002**
	1m and 3 ms	0.005**	0.35
	1m and 6 ms	0.0001**	0.68
	3ms and 6ms	0.0001**	0.09

Table 2: *Effect of different treatment modalities on the mean bacteriological results (PCR) at the study evaluation periods (Intra-group comparison).*

Time of Assessment	GI	GII	P value
Base line	31.60 ± 5.70	34.65 ± 1.07	0.15
1 month	28.49 ± 4.4	25.58 ± 5.33	0.18
3 months	30.99 ± 4.7	23.60 ± 3.74	0.0039**
6 months	36.21 ± 3.1	24.45 ± 3.48	0.0001**

Table 3*: Effect of different treatment modalities on the bacteriological results (PCR) at the study evaluation period (Inter-group comparison).*

*SD: Standard Deviation; F-test: For Analysis of Variance (ANOVA test); *Significant (P < 0.05). **Highly significant (P < 0.001) Non-significant (P > 0.05)*

Discussion

Results of the present study showed that, all the tested groups had approximately the same oral hygiene and periodontal conditions as evidenced by their mean PI and mean BOP scores at baseline prior to treatment.

The significant improvement in the mean PI and BOP scores in favor to group II as compared to group I at 6 months was explained by its antibacterial effect of ozone on the plaque microorganisms as SRP concurrently performed with ozone application enable oleozone to react directly with the planktonic bacterial allowing it to exert its optimal bactericidal effect with 24 hrs exposure. These results were supported by Kshitish D., *et al.* [12], Dhingra K., *et al.* [18], Montevecchi., *et al.* [19], Katti SS., *et al.* [20], Hayakumo S., *et al* [21].

Our results are in agreement with Nagayoshi., *et al*. [22] who found that treatment of ozonized water inhibited strongly the formation of dental plaque biofilm on decalcified human tooth, which may be due to the reduction of live bacteria in their in vitro study. These findings suggest that ozonized water with antiplaque activity might be effective as disinfectant solution.

Also, Fayek Y., *et al*. [23] revealed that BOP scores showed significant improvement at 8 weeks in favor of ozonated olive oil group as compared to SRP group in chronic periodontitis patient.

On contrary to our results, Al Habashneh., *et al.* [24] reported non- significant differences in BOP score between SRP and irrigation either with ozonized water or distilled water at 3 months in 41 patients with chronic peridontitis. These differences may be explained by the different concentration of ozone used, methodology of application and the drug form in which the oleozone gel have the advantage of prolonged retention in cells.

Group II showed a maintained PPD reduction and CAL gain till the end of the study (6months). These can be explained by its striking anti-hypoxic features of ozone brings about the rise of pO2 in tissues and improves transportation of oxygen in the blood, which results in change of cellular metabolism activation of aerobic processes (glycolysis, Krebs cycle, β-oxidation of fatty acids) and use of energetic resources. It also prevents formation of erythrocytes aggregates and increases their contact surface for oxygen transportation, thus stimulating the circulation and revitalizing organic functions [25].

Moreover, ozone was found to increase the distend ability of the membrane of the red blood corpuscles, leading to an increase the blood flow even through the ischemic tissues, hence provides tissues with nutrients, oxygen, inflammatory cytokines, and white blood cells needed for healing. It also increases washing action of the waste products out of the tissues and increases the oxygen carrying capability of the red blood corpuscles [25].

Several studies showed significant improvements in PDD and CAL gain after ozone therapy in different forms either gaseous or water or gas as an adjunct to subgingival debridement for periodontal treatment [20,21,26,27].

Contradictory to our results, Al Habashneh., *et al*. [24] showed that irrigation with ozonated water as an adjunctive therapy to SRP in chronic periodontitis produces no statistically significant benefit compared with SRP plus distilled water irrigation.

Of interest, the results of group III (SRP+oleozone gel) may be explained by the finding that SRP is effective in altering the flora. Besides, the use of oleozone can delay the repopulation of bacteria as it reduce the virulence of certain periodontal pathogen through its antimicrobial action on cells by damaging its cytoplasmic membrane via ozonolysis of dual bonds and induction of modification in the intracellular contents (oxidation of proteins, loss of organelle function) [28].

Our results were in agreement with Huth., *et al.* [29], Fayek Y., *et al.* [23], Montevecchi., *et al.* [19], Yılmaz S., *et al.* [27], Hayakumo S., *et al.* [21] and Hayakumo S [30]. who establish the promising results obtained for the O3-therapy against the opportunistic pathogens suggesting its potential applicability for periodontal treatment.

On contrary, Kshitishand Laxman [12], investigated the anti-bacterial effect of irrigation with ozonated water versus chlorohexidine gluconate (CHX) in the treatment of chronic periodontitis by using PCR analysis concluding that by using ozone and CHX, there was no antibacterial effect on *P. gingivalis* and T. *forsythia*. The possible explanation for the variation from that study results may be due to different concentration, viscosity of applied ozone form and different study design.

Unfortunately, non-significant recolonization at 6 months in group II may originate from microbiota in the supragingival plaque which proliferate matures and then spread subgingivally. Moreover, periodontal pathogen frequently colonizes oral mucosa, tongue dorsum and other oral niches may translocate from non-periodontal site to recolonize the periodontal pocket.

Also, Hosagi and Duncan [31] showed that *P. gingivalis* possesses protective mechanism to oxidative stress generated by toxic reactive oxygen species (ROS) through up-regulation of anti-oxidant genes resulting in the detoxification of ROS and peroxides. The robustness of this bacterium is not only due to the strong oxidative stress mechanisms that protect its DNA, proteins and lipid layer from oxidative damage, but also because of its excellent DNA repair process that accomplishes an efficient and precise removal of deleterious lesions.

It is worth mentioning that in spite of recolonization of the bacteria in both groups II and group III at 3 and 6 months, there was a maintained PPD reduction and CAL gain which is in consistence with Sánchez GA., *et al*. [32] evaluate the relation between the clinical parameters (PPD-CAL-BOP-PI) and level of *A. actinomycetemcomitans* and *P. gingivalis* (using PCR) at 30 and 60 days after non-surgical periodontal therapy. They concluded that *P. gingivalis* is present in periodontal pocket as well as healthy gingival margins. Furthermore, clonal heterogeneity of subpopulation of *P. gingivalis*, with both high and low levels of pathogenicity has been suggested to exist among periodontal pathogens harbored by individuals with negligible slight or even severe periodontal destruction. Therefore, there was a lack of association between *P. gingivalis* copies/ml in sub gingival plaque samples and severity of clinical signs of the disease.

Conclusion

The adjunctive topical subgingival application oleozone gel to one-session conventional mechanical therapy can provide clear cut improvements in the clinical, bacteriological and immunological parameters over the traditional treatment alone, which may reduce the need for additional treatment that could be periodontal surgery. Oleozone gel represents a promising natural product in both adjunctive and prophylactic treatments of chronic inflammatory periodontal diseases that could be re-applied without any expected adverse effect.

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