



The effects of subgingival application of ozonated olive oil gel in patient with localized aggressive periodontitis. A clinical and bacteriological study

M.Y.M. Shoukheba*, Sh.A. Ali

Oral Medicine, Periodontology, Oral Diagnosis and Radiology Department, Faculty of Dentistry, Tanta University, Egypt

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Abstract

This study evaluates the effect of subgingival application of ozonated olive oil gel as an adjunct to scaling and root planing (SRP) in aggressive periodontitis.

Material & methods: Thirty patients were randomly selected and equally divided into: Group I received SRP only, group II received SRP and ozonated olive oil gel (*Oxactiv*). Subgingival application of ozone gel was performed following initial SRP and at 7, 14 and 21 days. Clinical measurements included pocket depth (PD), plaque index (PI), gingival index (GI), bleeding on probing (BOP) and clinical attachment level (CAL). Real time PCR was carried out to determine the effect of the treatment on both *Aggregatibacter actinomycetemcomitans* (*Aa*) and *Porphyromonas gingivalis* (*Pg*). Clinical measurements and Plaque samples for PCR were recorded at baseline, one, three and six months after treatment.

Results: The results showed improvement in all clinical parameters in (group II) which was maintained up to six months ($P < 0.05$). However, this improvement was best following one month but gradually decrease at 3 and 6 months. Whereas SRP alone resulted in a significant improvement only up to one month for BOP, PPD and CAL parameters and up to three months for the PI and GI scores as compared to baseline values.

Microbiological results: Revealed significant reduction of the mean *Pg* and *Aa* DNA copies at 1 and 3 months for (group II), whereas group I resulted in slight reduction up to 1 month only followed by gradual increase reaching baseline values. There was no significant difference between groups at three and six months regarding *Pg* DNA copies. There was a significant difference between groups at the one and three months periods in term of number of *Aa* copies ($P < 0.001$, $P < 0.05$ respectively).

* Corresponding author.

E-mail addresses: smalakyousefmohamed@yahoo.com, malak.mohamed@dent.tanta.edu.eg, osama_nada1972@yahoo.com (M.Y.M. Shoukheba), smalakyousefmohamed@yahoo.com, shyreen.moawad@dent.tanta.edu.eg (Sh.A. Ali).

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Conclusion: The study concluded that (*Oxactiv*) gel could be a promising adjunct to SRP in the treatment of aggressive periodontitis.

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Keywords: Ozone; Aggressive periodontitis; SRP

1. Introduction

Currently, ozone therapy is gaining popularity as a modern non-invasive method of treatment [1]; it is a powerful oxidizing agent with a high antimicrobial power against oral pathogens, without resistance development has been reported not only for gaseous ozone [2,3], but also for ozone in aqueous [4–7]. It is used in various treatment modalities in field of medicine, dentistry, veterinary, food industry, and water treatment. Ozone is being discussed in dentistry as a possible alternative oral antiseptic agent. Its A high level of biocompatibility to fibroblasts, cementoblasts, and epithelial cells [8,5,6,9] suggests its use against oral infectious diseases [3,10].

Ozonated olive oil (*Oxaktiv*) is 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 [11]. Due to the germicidal action of ozone, as well as its oxygenating power, that favors tissue regeneration, it was applied in the treatment of alveolitis following surgical extraction of the lower third molar [12].

Aggressive periodontitis encompasses distinct types of periodontitis that affect people who are otherwise appear healthy [13,14]. It is characterized by familial aggregation and there is a rapid rate of disease progression [15]. An important factor in the pathogenesis of AgP is the infection of the periodontium by pathogenic bacteria which acts as a primary etiologic agent in this disease [16]. Individuals with aggressive periodontitis are usually infected by a gram-negative anaerobic rod species [17]. Its etiology is highly correlated to the presence of *Aggregatibacter actinomycetemcomitans* [18,19], host response defects [20,21], and possibly to genetic inheritance [22,23].

Moreover, studies demonstrated that there are different microbial associations in subgingival plaques *Tannerella forsythia* (*T. forsythia*), *Porphyromonas* seem to be related to the progression of periodontal destruction in aggressive periodontitis [24,25]. Due to the complexity of the aggressive periodontal diseases with regard to systemic factors, immune defects and microbial

flora, control of the disease may not be possible in all instances. Additionally, it has the potential to cause tooth mobility and pathological tooth movement, thus effective treatment and management of those affected are necessary to slow the disease progression [26–28].

The topical application of ozone has been used in management of periodontitis but the literature regarding the direct effect of ozone on the oral tissues is still obscure [29]. This stimulated the idea of the present work in studying the effect of using ozonated olive oil gel as an adjunctive treatment to scaling and root planing as compared to scaling and root planing alone in the treatment of localized aggressive periodontitis.

2. Material and methods

In the present study, 30 subjects with localized aggressive periodontitis (21 females and 9 males) whose ages ranged from 21 to 30 years were selected from Periodontology Department clinic, Faculty of Dentistry, Tanta University according to the criteria outlined by Carranza (1998) [30]. Panoramic X ray was done for each patient to confirm the diagnosis. All the selected subjects were ascertained to be in good general health with no history of any systemic disease (hyperthyroidism, glucose-6-phosphate-dehydrogenase deficiency, severe myasthenia) [31] and no history of antibiotic therapy, oral prophylaxis, or periodontal surgery during the last six months. Pregnant or lactating females as well as smokers were excluded, and a written consent was signed by the patients.

2.1. Clinical parameters

The clinical examination was carried out with a manual probe (Williams 14 W) from the deepest pocket for each patient. The following clinical measurements were recorded: Plaque index (PI), assessed according to (Silness and Loe 1964) [32], Gingival index (GI), according to (Loe and Silness 1963) [33], Bleeding on probing (BOP) according to (Ainamo and Bay, 1975) [34], Probing depth (PD) according to (Ramfjord, 1967) [35], Clinical attachment level (CAL) according to (Ramfjord, 1967) [35]. Baseline

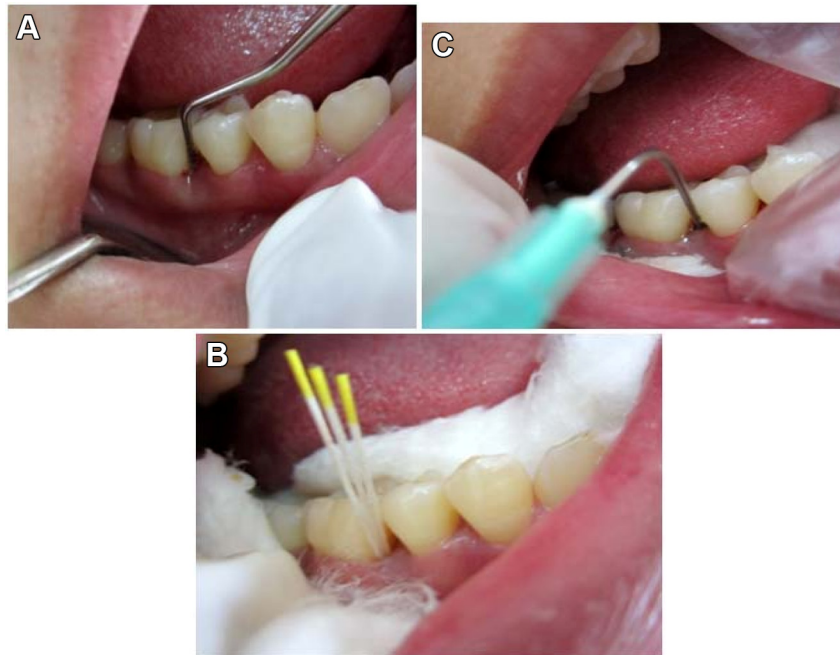


Fig. 1. A): Baseline probing pocket depth measurement in patient with generalized aggressive periodontitis. B: Subgingival plaque sample collected using paperpoint. C: Subgingival application of Ozonated olive oil gel using 10 ml syringe.

measurements were taken at day zero before treatment; clinical measurements were taken at 1, 3, and 6 months respectively without any additional treatment performed to the patients.

Patients were selected randomly by coin toss process, of which 15 patients received oral hygiene instructions and SRP of all teeth Group I (Control group) and 15 patients received oral hygiene instructions, SRP of all teeth in addition to subgingival application of ozonated olive oil gel *Oxactiv gel*¹.

2.2. Group II (test group)

All participants received full written and verbal information about the study and signed the informed consent form and patients who did not get a complete response were referred for other therapies including surgery with regenerative techniques.

2.3. Gel application

Patients qualified for this study were undergo a full mouth scaling using ultrasonic scalers and hand instrumentation followed by subgingival application of

Oxactiv gel in the deepest selected periodontal pocket using disposable 10 ml plastic syringe. The selected teeth were isolated carefully with cotton rolls and thoroughly dried and the gel was applied carefully subgingivally and interproximally until excess gel was observed from the gingival margin. This procedure was repeated for all teeth to be treated (Fig. 1C). Excess gel was removed with a cotton roll and patients were instructed not to eat, drink, or rinse for at least 30 min. Patients were also instructed to refrain from chewing hard or sticky foods, brushing near the gel treated site or using inter-dental aids. Instructions with supragingival brushing at the site of application were given. Patients who used antibiotic during the study period were excluded from the study to be sure that the outcome of therapy was not affected. Adverse effects were noted and any supragingival deposits were removed at recall visits. Gel application was performed after initial SRP and at 7, 14 and 21 days. Reinforcement of oral hygiene instructions at each appointment has been done.

2.4. Microbiological examinations

Prior to the scaling and root planing and gel application, a baseline subgingival plaque sample was taken

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from the deepest periodontal pocket (≥ 6 mm probing depth) for Real time PCR (RT.PCR) analysis.

The sampling site was isolated with cotton rolls before taking the sample. The supragingival plaque was removed with a sterile curette and cotton gauze. Subsequently, a subgingival plaque sample was obtained using three sterile paper points, inserted into the pocket until resistance was met or the paper points bent [36]. They were each kept in place for 10 s and then transferred to one ml reduced Ringer's solution phosphate buffer solution Fig 1B.

2.5. Preparation for RT.PCR

Subgingival samples were suspended into 500 μ l of TE buffer (10 mM Tris-hydrochloride, 1 mM EDTA, pH 8) and were sent to the microbiological laboratory within 24 h of collection, where they were subjected to real time PCR polymerase chain reaction (RT.PCR) analysis to quantify *Aggregatibacter actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg). Samples were repeated at one, three and six months after treatment.

2.5.1. Isolation of DNA from subgingival plaque samples

DNA was extracted with the Qiagen extraction mini kit (Qiagen, Valencia, CA). Quantitative real-time PCR of *P. gingivalis* was carried out according to Sanz et al., (2004) [37] using ABI Prism 7000 Sequence Detection instrument (Applied Biosystems).

2.5.2. Procedures

- 1) After DNA extraction, PCR reaction mix was prepared for each sample separately. For 25 μ l reaction, containing 200 mg DNA, 200 nm/L

deoxynucleotide triphosphate, 2 mm/L $MgSO_4$, 140 nm/L of each primer (Table 1).

- 2) The solutions were mixed by gentle pipetting up and down then capped in the tubes which were centrifuged briefly to spin down the content and eliminate any air bubbles.
- 3) The appropriate volume of each reaction mixture was transferred to wells of an optical plate with an optical adhesive cover.
- 4) Tubes were centrifuged again to spin down the content and eliminate any air bubbles and the reaction plate was placed in the instrument.
- 5) The thermal cycler conditions were set by heating at 50 °C for 2 min, 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s, 60 °C for 1 min, and 72 °C for 1 min, with a final step for 10 min at 72 °C using thermal mixer.
- 6) The reaction volume at 25 μ l was setted and the run started.
- 7) The detection limit of the real-time PCR was assessed by determining the C_t values of serial 7-fold dilutions of purified genomic DNA from *P. gingivalis* and *A. actinomycetemcomitans* strain. A standard curve prepared with these dilutions was used in every experiment.

2.5.3. Data analysis

Relative quantification was done automatically by ABI Prism 7000 Sequence Detection system using standard and amplification curves.

2.6. Statistical analysis

All the results were tabulated and statistically analyzed using Statistical package for social science (SPSS version 12). Intragroup comparison was done using paired *t*-test. The difference between groups was statistically analyzed using independent sample *t*-test.

3. Results

3.1. Clinical results

Both treatment modalities were well tolerated by the patients without any complications. The baseline measurements showed no significant differences among the two treatment modalities for any of the clinical parameters measured ($P > 0.05$). The improvement in all clinical parameters was markedly observed in the ozone treated group (group II) which was maintained up to six months ($P < 0.05$) except for

Table 1

The sequence of primer and TaqMan probes used for real-time PCR.

A. actinomycetemcomitans

Forward -F,CAAGTGTGATTAGGTAGTTGGTGGG
M75039

Reverse -R,CCTTCCTCATCACCGAAAGAA

TaqMan probe ATCGCTAGCTGGTCTGAGAGGATGGCC

P. gingivalis

Forward -F, 5'-GCGCTCAACGTTTCAGCC-3'
(base pair 612–628)

Reverse -R, 5'-CACGAATTCCGCCTGC-3'
(base pair 664–679)

TaqMan probe 5'-CACTGAAGTCAAGCCCGGCGAGTTTCAA-3',
(base pairs 634–660).

Table 2

The effect of both treatments on the mean values of plaque index (PI) among the study groups at baseline, 1, 3 and 6 months after treatment.

Plaque index	Group I	Group II	<i>t</i>	<i>P</i>
<i>At baseline:</i>				
Mean ± SD	1.712 ± 0.05	1.710 ± 0.02	0.582	0.565 ^{ns}
<i>At 1 month:</i>				
Mean ± SD	1.19 ± 0.72	0.94 ± 0.07	3.516	0.002**
Paired <i>t</i>	7.23	40.06		
<i>P</i>	0.000***	0.000***		
<i>At 3 month:</i>				
Mean ± SD	1.49 ± 0.16	1.33 ± 0.14	2.910	0.007**
Paired <i>t</i>	4.91	10.14		
<i>P</i>	0.000***	0.000***		
<i>At 6 month:</i>				
Mean ± SD	1.72 ± 0.17	1.60 ± 0.13	2.168	0.039*
Paired <i>t</i>	0.000	3.42		
<i>P</i>	1.00 ^{ns}	0.004*		

bleeding on probing (up to 3 months only). Whereas SRP alone resulted in a significant improvement only up to one month for BOP, PPD and CAL parameters and up to three months for the PI and GI scores as compared to baseline value. There were statistically significant differences between the two groups throughout the study evaluation periods in favor of group II, Tables 2 and 3, Fig. 2, 3.

Table 4, Fig. 4 illustrate the effects of the treatments on BOP, test group resulted in significant reduction of BOP index up to 3 months ($P < 0.001$) while control group resulted in significant reduction at one month only ($P < 0.001$) followed gradual increase reached nearly to the baseline value ($P > 0.05$) for both groups.

Compared to baseline data, the test group showed statistically significant reduction of PD and CAL gain

Table 3

The effect of both treatments on the mean values of gingival index (GI) among the study groups at baseline, 1, 3 and 6 months after treatment.

Gingival index	Group I	Group II	<i>t</i>	<i>P</i>
<i>At baseline:</i>				
Mean ± SD	1.65 ± 0.12	1.64 ± 0.14	0.281	0.781 ns
<i>At 1 month:</i>				
Mean ± SD	1.38 ± 0.21	1.07 ± 0.08	6.510	0.000***
Paired <i>t</i>	4.317	17.009		
<i>P</i>	0.001***	0.000***		
<i>At 3 month:</i>				
Mean ± SD	1.58 ± 0.07	1.42 ± 0.21	2.871	0.008**
Paired <i>t</i>	2.32	3.912		
<i>P</i>	(0.036)*	0.002**		
<i>At 6 month:</i>				
Mean ± SD	1.70 ± 0.093	1.51 ± 0.18	3.550	0.001**
Paired <i>t</i>	−1.418	2.356		
<i>P</i>	0.178 ^{ns}	0.034*		

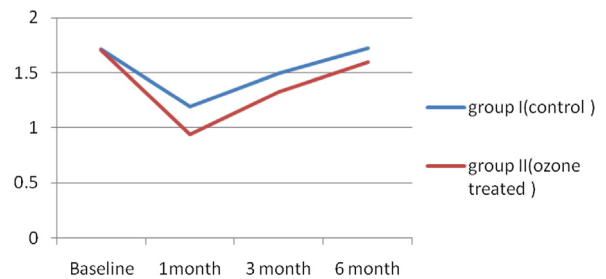


Fig. 2. Plaque index score (PI) among the study groups at different follow up periods.

at one month and three months after therapy ($P < 0.001$) followed by increase in the mean PD and CAL reaching nearly to the baseline values. On the other hand control group, showed statistically significant PD reduction and CAL gain at one month $P < 0.001$ followed by gradual increase reaching slightly above the baseline value at 6 months evaluation period. Tables 5 and 6, Figs. 5, 6. Comparison between the two treatment modalities showed that there is statistically significant differences between the tested and the control group at one and three months evaluation periods $P < 0.001$ and insignificant difference at six month $P > 0.05$, Tables 5 and 6.

3.2. Microbiological results

3.2.1. Quantification of *P. gingivalis*

Baseline data shows no significant differences between the two treatment groups $P > 0.05$.

3.2.2. Group I (SRP only)

One month post treatment, there was mild significant reduction in the mean number of *P.g* DNA copies $P < 0.05$ mean number slightly decreased to ($2.06 \times 10^3 \pm 0.25 \times 10^3$) copies as compared to ($2.24 \times 10^3 \pm 0.33 \times 10^3$) copies at baseline. On the other hand, at 3 and 6 months the mean number of

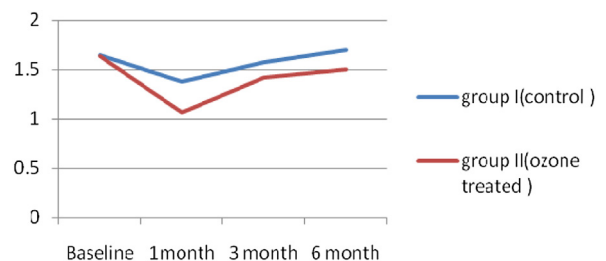


Fig. 3. Gingival index score (GI) among the study groups at different follow up periods.

Table 4

The effect of both treatments on the mean values of bleeding on probing (BOP) among the study groups at baseline, 1, 3 and 6 months after treatment.

Bleeding on probing	Group I	Group II	<i>t</i>	<i>P</i>
<i>At baseline:</i>				
Mean $\pm$ SD	74.23 $\pm$ 3.95	74.53 $\pm$ 3.11	−0.231	0.819 ^{ns}
<i>At 1 month:</i>				
Mean $\pm$ SD	43.60 $\pm$ 12.91	31.33 $\pm$ 6.39	3.296	0.003**
Paired <i>t</i>	8.34	21.59		
<i>P</i>	0.000***	0.000***		
<i>At 3 month:</i>				
Mean $\pm$ SD	71.07 $\pm$ 5.16	54.07 $\pm$ 6.99	7.574	0.000***
Paired <i>t</i>	1.78	11.39		
<i>P</i>	0.096 ^{ns}	0.000***		
<i>At 6 month:</i>				
Mean $\pm$ SD	72.87 $\pm$ 4.17	72.66 $\pm$ 2.71	0.156	0.877 ^{ns}
Paired <i>t</i>	1.24	3.06		
<i>P</i>	0.232 ^{ns}	0.008 ^{ns}		

DNA copies increased gradually reaching slightly above baseline value ($2.14 \times 10^3 \pm 0.36 \times 10^3$, $2.366 \times 10^3 \pm 0.399 \times 10^3$ respectively) with no significant difference $P > 0.05$ as shown in Table 7 Fig. 7.

3.2.3. Group 2 (ozone treated group)

Three months postoperatively, ozone treated group showed decrease in mean number of the *P.g* DNA copies to ($1.74 \times 10^3 \pm 0.23 \times 10^3$) and ($2.106 \times 10^3 \pm 0.25 \times 10^3$) copies at one and three months respectively which is a statistical significant reduction compared to baseline values ($2.28 \times 10^3 \pm 0.32 \times 10^3$) $P < 0.001$ and $P < 0.05$ respectively. On the other hand, at 6 months the mean number of DNA copies increased to ($2.24 \times 10^3 \pm 0.32 \times 10^3$) which is statistically insignificant $P > 0.05$.

Intergroups comparison, showed that, there was a statistically significant difference at one month, ($P < 0.01$) where group II resulted in better reduction in

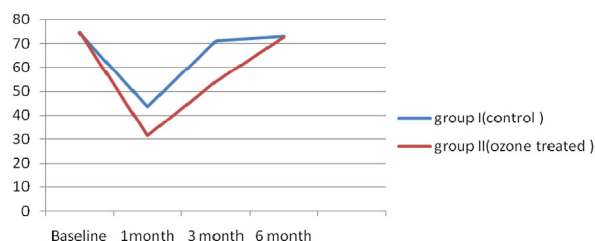


Fig. 4. Bleeding on probing score (BOP) among the study groups at different follow up periods.

Table 5

The effect of both treatments on the mean values of probing pocket depth (PPD) among the study groups at baseline, 1, 3 and 6 months after treatment.

Probing pocket depth	Group I	Group II	<i>t</i>	<i>P</i>
<i>At baseline:</i>				
Mean $\pm$ SD	5.87 $\pm$ 0.27	5.85 $\pm$ 0.29	0.257	.799 ^{ns}
<i>At 1 month:</i>				
Mean $\pm$ SD	5.36 $\pm$ 0.42	4.60 $\pm$ 0.45	5.565	0.000***
Paired <i>t</i>	6.48	10.04		
<i>P</i>	0.000***	0.000***		
<i>At 3 month:</i>				
Mean $\pm$ SD	5.74 $\pm$ 0.26	5.04 $\pm$ 0.33	6.415	0.000***
Paired <i>t</i>	1.883	6.7		
<i>P</i>	0.081 ^{ns}	0.000***		
<i>At 6 month:</i>				
Mean $\pm$ SD	5.84 $\pm$ 0.20	5.61 $\pm$ 0.37	2.040	0.051 ^{ns}
Paired <i>t</i>	0.598	2.419		
<i>P</i>	0.560 ^{ns}	0.03*		

the number of *Pg* copies. On the other hand, there is no significant difference between groups at three and six months $P > 0.05$. Table 7, Fig. 7.

3.3. Quantification of *A. actinomycetumcomitans*

Baseline data shows no significant differences between the two treatment groups $P > 0.05$.

3.3.1. Group 1 (SRP only)

At one month post treatment, there is slight significant decrease in the mean number of *A.a* DNA copies to ($3.27 \times 10^5 \pm 0.26 \times 10^5$), which is statistically

Table 6

The effect of both treatments on the mean values of clinical attachment level (CAL) among the study groups at baseline, 1, 3 and 6 months after treatment.

Clinical attachment loss	Group I	Group II	<i>t</i>	<i>P</i>
<i>At baseline:</i>				
Mean $\pm$ SD	5.3 $\pm$ 0.20	5.38 $\pm$ 0.34	−0.836	0.410 ^{ns}
<i>At 1 month:</i>				
Mean $\pm$ SD	4.98 $\pm$ 0.23	4.65 $\pm$ 0.26	3.694	0.001**
Paired <i>t</i>	4.25	6.70		
<i>P</i>	(0.001)**	0.000***		
<i>At 3 month:</i>				
Mean $\pm$ SD	5.33 $\pm$ 0.26	4.86 $\pm$ 0.36	4.008	0.000***
Paired <i>t</i>	−0.495	3.94		
<i>P</i>	0.628 ^{ns}	0.001**		
<i>At 6 month:</i>				
Mean $\pm$ SD	5.26 $\pm$ 0.24	5.28 $\pm$ 0.31	−0.179	0.860 ^{ns}
Paired <i>t</i>	0.509	3.623		
<i>P</i>	0.619 ^{ns}	0.003**		

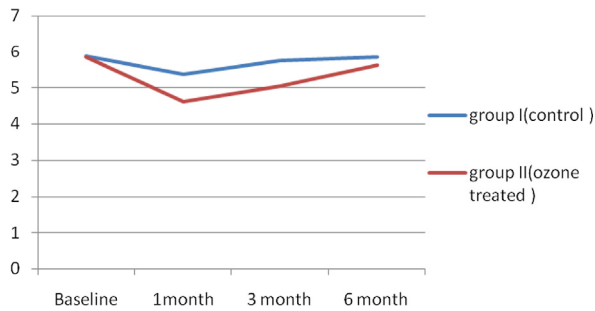


Fig. 5. Probing pocket depth (PPD) among the study groups at different follow up periods.

significant $P < 0.05$ compared to its baseline mean number ($3.56 \times 10^5 \pm 0.28 \times 10^5$). At three and six months, the mean number recorded ($3.66 \times 10^5 \pm 0.219 \times 10^5$, $3.9310^5 \pm 1.42 \times 10^5$ respectively) which is statistically insignificant as compared to the baseline value $P > 0.05$ as shown in Table 8, Fig. 8.

3.3.2. Group 2 (ozone treated)

There was statistically significant reduction in the mean number of DNA copies at one and three months evaluation periods ($1.016 \times 10^5 \pm 0.67 \times 10^5$, $2.31 \pm 0.72 \times 10^5$) ($P < 0.001$) compared to its baseline value ($3.55 \times 10^5 \pm 0.27 \times 10^5$). On the other hand, at six months there is insignificant increase in the mean number of DNA copies to ($3.57 \times 10^5 \pm 0.24 \times 10^5$) ($P > 0.05$), this was recorded in Table 8, Fig. 8.

3.4. Intergroups comparison of the results

There was a statistically significant difference at one and three months ($P < 0.001$) where group II resulted in better reduction in the number of *A.a* copies. On the other hand, no significant difference between groups at six months ($P > 0.05$) was observed.

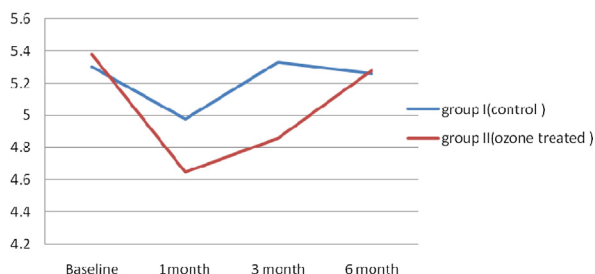


Fig. 6. Clinical attachment loss (CAL) among the study groups at different follow up periods.

Table 7

The results of real time PCR for *P. gingivalis* at baseline, 1, 3 and 6 months after treatment.

Pg DNA copies ($\times 10^3$)	Group I	Group II	<i>t</i>	<i>P</i>
<i>At baseline:</i>				
Mean $\pm$ SD	2.24 $\pm$ 0.33	2.28 $\pm$ 0.32	−0.317	0.0754 ^{ns}
<i>At 1 month:</i>				
Mean $\pm$ SD	2.06 $\pm$ 0.25	1.74 $\pm$ 0.23	3.014	0.006**
Paired <i>t</i>	2.416	5.518		
<i>P</i>	0.030*	0.000***		
<i>At 3 month:</i>				
Mean $\pm$ SD	2.14 $\pm$ 0.36	2.106 $\pm$ 0.25	0.093	0.927 ^{ns}
Paired <i>t</i>	0.655	2.300		
<i>P</i>	0.523 ^{ns}	0.036*	0.126	0.518 ^{ns}
<i>At 6 month:</i>				
Mean $\pm$ SD	2.366 $\pm$ 0.399	2.24 $\pm$ 0.32		
Paired <i>t</i>	−2.416	0.690		
<i>P</i>	0.241 ^{ns}	0.500 ^{ns}		

4. Discussion

The initiation and progression of periodontitis is caused by different bacterial accumulations in the subgingival pockets. Elimination of the subgingival microflora may be achieved mechanically. However the effectiveness of this method is limited by such factors as concave tooth surfaces, margins of restorations and inaccessibility of periodontal pockets so mechanical method can also be supplemented by antimicrobial agent [38]. Aggressive periodontitis is a progressive periodontal disease characterized by loss of bone and periodontal support for special teeth in adolescents and young adults [39]. It was also proved that certain bacterial types exist in the periodontal pockets of aggressive periodontitis patients and these bacteria have been associated with active lesions in those patients [40].

The periotherapy takes three essential directions, preventive for the non established periodontitis, surgical or the non-surgical remedy for the established

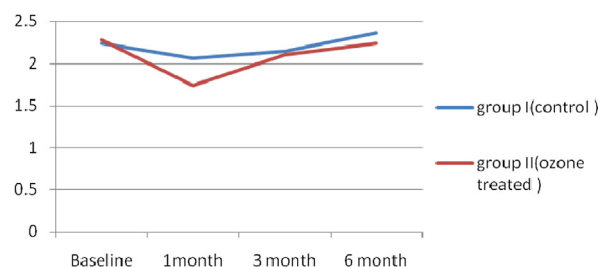


Fig. 7. Results of real time PCR for *P. gingivalis* among groups 1&2 at different follow up periods.

Table 8

The results of real time PCR for *A. a* at baseline, 1, 3 and 6 months after treatment.

Aa DNA copies ($\times 10^5$)	Group I	Group II	T	P
<i>At baseline:</i>				
Mean $\pm$ SD	3.56 $\pm$ 0.28	3.55 $\pm$ 0.27	-0.095	0.925 ^{ns}
<i>At 1 month:</i>				
Mean $\pm$ SD	3.27 $\pm$ 0.26	1.016 $\pm$ 0.67	20.767	0.000***
Paired t	2.620	11.741		
P	0.03*	0.000***		
<i>At 3 month:</i>				
Mean $\pm$ SD	3.66 $\pm$ 0.219	2.31 $\pm$ 0.72	8.268	0.000***
Paired t	-0.953	5.59		
P	0.357 ^{ns}	0.000***	0.972	0.340 ^{ns}
<i>At 6 month:</i>				
Mean $\pm$ SD	3.93 $\pm$ 1.426	3.57 $\pm$ 0.24		
Paired t	-1.032	-0.208		
P	0.319 ^{ns}	0.838 ^{ns}		

periodontitis (26). The surgical periotherapy based upon the ideology of excision of the infected tissues and modify the environment to get the best condition for rehabilitating process with rigorous maintenance follow up [41]. The non-surgical periotherapy based upon the bacteriological findings and on the susceptibility of the causative microorganisms to the antimicrobial drugs given [42].

The aim of this study was to investigate the antimicrobial effectiveness of a commercially available ozonated olive oil (*Oxactiv*) against *A.a* and *P. gingivalis* as a causative organisms in localized aggressive periodontitis which is considered as challenging treatment to the periodontist.

Ozone therapy is gaining popularity in various treatment modalities in the field of medicine, dentistry [5]. In vitro studies have reported the antimicrobial effect of ozonated water specially against *Candida albicans*, adhering to acrylic denture plates [7], on *Enterococcus faecalis* [43], and *Streptococcus mutans*

[6] and periodontopathic bacteria such as *Aa* and *Pg* in vitro [44]. Ozone reacts with various chemical compounds in two different and coexisting modes, one involving direct reactions of molecular ozone and the other a free radical-mediated reaction [45]. Both these mechanisms may be involved in the destruction of bacteria by ozone.

There are several ways of delivering ozone, when it dissolves in water, it becomes highly unstable and rapidly decomposes through a complex series of chain reactions, so it cannot be stored [46,47]. In contrast, when it is dissolved in an oil base, it has a life span that could be measured in years. It chemically reacts with oil, and forms long complex molecules [1,11]. Hence in this study ozonated olive oil gel was selected over ozonated water because the application of gels was found to provide a long stay in the oral cavity, adequate drug penetration, high efficacy and acceptability [48], this is in addition to the action of omega 3 in olive oil which have potential benefits as a host modulatory agent in the adjunctive management of periodontitis [49].

Zaki et al., 2012 [29], found that application of ozone for more than one month resulted in degeneration of lamina propria and the only normal histopathological samples were obtained when ozone was applied once per week for one month or twice per week for one month. So in this study ozone was applied once per week for one month.

Real time PCR was used since it is an important tool for the rapid, sensitive, and specific detection of bacterial pathogens [50]. However, conventional PCR does not allow accurate quantification and thus misses important diagnostic aspects [51]. The objective of this study was to assess the clinical and antimicrobial effect of ozonized water in management of aggressive periodontitis.

Results of the present study revealed that ozone treated group (group II) showed improvement in all clinical parameters in which was maintained for six months ($P < 0.05$) except for BOP the improvement was up to 3 months only. Whereas SRP alone resulted in a significant improvement only up to one month for BOP, PPD and CAL parameters and up to three months for the PI and GI scores. From the bacteriological point of view results revealed statistically significant reduction of the mean *Pg* and *Aa* DNA copies at 1 and 3 months for ozone treated group II, whereas group I resulted in slight significant reduction up to 1 month only followed by gradual increase up to baseline value. There was no significant difference between groups at three and six months regarding *Pg* DNA copies. There

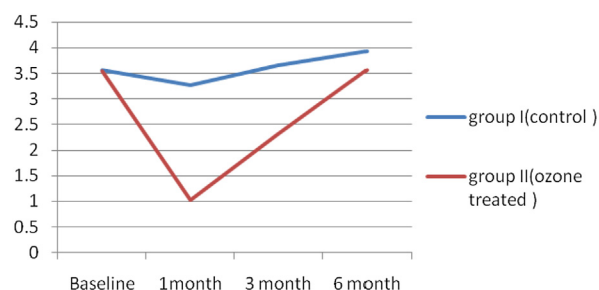


Fig. 8. Results of real time PCR for *Aggregatibacter actinomycetemcomitans* (*Aa*) 1&2 at different follow up periods.

were statistically significant differences between groups at the one and three months periods regarding the number of (*Aa*) copies ($P < 0.001$, $P < 0.05$ respectively).

Study done by Sorokina & Lukinych (1997) [52] using subgingival irrigation with ozonized water in combination with professional measures of oral hygiene, plaque formation was reduced due to pronounced anti-inflammatory effects on the periodontium after using irrigation of periodontal pockets with ozone.

Ozone was found to have a potent antibacterial effect explained by the fact that it causes disruption of the envelope integrity through peroxidation of phospholipids [53,12]. Additionally, the inhibitory effect of ozone on NF-kappa B system making it a potent anti-inflammatory agent and can stop disease activity [9]. Moreover, Ozone reacts with various chemical compounds in aqueous systems in two different and coexisting modes, one involving direct reactions of molecular ozone and the other a free radical-mediated reaction [45]. Both these mechanisms may be involved in the destruction of bacteria by ozone.

Kshitish and Laxman (2010) [38] demonstrated, higher percentage of plaque index (12%), gingival index (29%) and bleeding index (26%) reduction was observed using ozone irrigation. Additionally, Patel et al. (2012) [54] showed that, the adjunctive use of the Ozonated olive oil gel (OZO) with SRP in treatment of chronic periodontitis resulted in a significant improvement ($P < 0.001$) of clinical parameters as well as microbiological parameters over the time and in comparison to the control groups. The OZO as monotherapy also showed a significant improvement ($P < 0.001$) in clinical parameters as well as microbiological parameters over the time without any documented side effects. However, there was a significant increase ($P < 0.05$) in dentinal hypersensitivity following OZO as an adjunct to scaling and root planing therapy.

The improvement due to scaling and root planing is in agreement with (Savitt and Socransky 1984, Pattison and Pattison, 2002) [55,56] and can be explained by, that SRP remove the microbiologically contaminated cementum layer and to eliminate and reduce the number of pathogenic microorganisms in periodontal pocket to level below those required to induce the disease. These changes in the microbiota are accompanied by reduction or elimination of inflammation clinically. Additionally, scaling and curettage procedures, resulted in healing and improvement of pocket depth result from the ability of the tissues to form thin junctional epithelium and the resistance of the tissue to

the probe penetration since the contaminated cementum layer was removed [57].

However, SRP does have limitations including the inability to adequately instrument deep periodontal pockets and as well as to reduce the total bacterial load and change the environmental conditions of these microbial niches within the tissues lining of the periodontal pocket. Hence, removal of deposits and organisms from these locations may require surgical intervention or the use of antimicrobial agents [58].

The results agreed with Nagayoshi et al. (2002) [59] who found that ozonized water should be useful in reducing the infections caused by oral microorganisms in dental plaque. And also agreed with Kshitish and Laxman (2010) [38] who reported that, using ozone has a powerful ability to inactivate periodontal microorganisms and is appreciable against *Aa* as compared to Chlorhexidine.

Moreover, the in vitro study done by Eick et al. (2011) [60] reported that ozone has a strong antibacterial activity against putative periodontopathogenic microorganisms and suggested that it may have potential effect as an adjunctive application to SRP in periodontitis patients. Additionally, this result was supported an in vitro by Nagayoshi et al. (2004) [5] who showed that ozonated water (0.5–4 mg/l) was highly effective in killing both gram positive and gram negative oral microorganisms such as *Porphyromonas endodontalis*, *Porphyromonas gingivalis*. Moreover, Huth et al. (2011) [61] found significant reduction in periodontal pathogens namely *P. gingivalis*, *Parvimonas micra*, *Tannerella forsythia* on irrigation with gaseous/aqueous ozone as compared to 0.2%CHX. None of the agents could substantially reduce *A. actinomycetemcomitans* (*A.a*) count in biofilm cultures. On the contrary, an in vitro study by Müller et al. (2007) [62] reported that there is no success in reducing the microbiota on applying ozone.

However, the improvement didn't last over entire experimental period since the persistence of deep pocket favored recolonization of the putative periodontal pathogens. Accordingly, surgical management for the pockets is necessary for evaluation of microbial ecological niches. A more meaningful method is to compare the comprehensive surgical technique comparing the use of ozonated olive oil versus antibiotics in the treatment of deep pocket in aggressive periodontitis.

At the start of this study there was an expectation that the treatment will be not effective with some of the patients so an informed consent was given to the patient. Meanwhile, patients who did not get a complete response were referred for other therapies including surgery with regenerative techniques.

Considering the limitation of this study in terms of short-term of sample size, it can be concluded that local ozone application can serve as a potential atraumatic, promising antimicrobial agent to treat periodontal disease non-surgically, both for home care and for professional practice. It may serve as a good tool during supportive periodontal therapy. Further long time studies are required to adequately assess the concentration of ozone that is effective against pathogens. However it is required to determine the specific ozone concentration that is effective against anaerobic periodontopathogens.

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