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Clinical trial

EVALUATION OF BENEFITS FROM OZONE THERAPY IN PATIENTS WITH CHRONIC PERIODONTITIS

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Abstract

Aim: The study evaluated the effects of subgingival application of ozonated olive oil as adjuvant therapy to classic non-surgical conservative periodontal treatment.

Material and methods: Sixty patients with moderate and advanced periodontal disease were divided into two groups: group 1, in which only conservative periodontal treatment (scaling and root planning) was applied, and group 2, in which, in addition to the conservative treatment, ozonated olive oil (Ozone Gold-Germany, GmbH, 80 mg) was also applied. The subgingival application of ozonated olive oil was performed right after the conservative treatment and also, on days 7, 14 and 21. Clinical parameters included DPI, IGI, GBI, CAL. BANA test was performed to detect the presence of the three anaerobic periopathogenic bacterial species (*T. denticola, T. forsythia, P. gingivalis*) in subgingival dental plaque. Clinical parameters and BANA test were recorded at the beginning, before starting conservative treatment and 1 month afterwards.

Results: The results indicated an improvement in all clinical parameters in both groups, with a statistical significance in the group in which ozonated olive oil was applied in addition to conservative periodontal treatment. The combined treatment showed a significantly greater success than the conservative one in terms of the presence of the three anaerobic periopathogenic bacterial species (*T. denticola, T. forsythia, P. gingivalis*).

Conclusion: Topical application of ozonated olive oil in addition to conservative periodontal treatment may serve as a potential atraumatic, promising antimicrobial agent in the non-surgical treatment of periodontal disease.

Keywords: ozonated olive oil, BANA test, conservative periodontal treatment

Introduction

Epidemiological research indicate that periodontal diseases are one of the most widespread diseases, affecting more than 90% of the world population.

They can occur as independent diseases (gingivitis), but sometimes, due to degenerative, atrophic and other processes, which can be dominant, they tend to affect the entire dental support apparatus. Periodontal diseases lead to gradual, anatomical and functional disintegration of tissues, resulting in gradual loosening and later loss of one or more teeth. Clinically, they are manifested by gum bleeding, progressive increase in the depth of periodontal pockets, loss of attachment and loss of alveolar bone[1-3].

Today it is known with certainty that in the etiology of these diseases, in addition to other local and general factors, the key role belongs to certain microorganisms from the dental plaque.

In vitro studies of microbial communities in dental biofilms highlight the importance of structural and physiological interactions between bacterial species in plaque. The latest analyses of more than 40 subgingival microorganisms in 13,000 plaque samples using the DNA hybridization methodology defined the so-called "complexes" of periopathogenic microorganisms. The red complex of bacteria, which it consists of *T. denticola, T. forsythia, P. gingivalis*, is of particular interest, because it is associated with bleeding during probing, which is an important clinical parameter for destructive periodontal diseases[4].

Anaerobic bacteria, as the most common residents in subgingival dental plaque, contain enzymes that can hydrolyze peptides. But only *P. gingivalis, T. denticola* and *T. forsythia* possess significant amounts of unique peptidases-type enzymes. Taking into account these unique characteristics, researchers from the University of Michigan developed a synthetic peptide, BANA (N-benzoyl-DL-arginine-2-napthylamide) which activity was originally described as a "trypsin-like protease" to detect the presence of the common enzyme[5].

The BANA test (N-benzoyl-DL-arginine-2-napthylamide) is a very simple and quick test, which is performed in 15 minutes and provides information on the presence of the three anaerobic periopathogenic bacterial species (*T. denticola, T. forsythia, P. gingivalis*) in the subgingival dental plaque. The test results can be read immediately and results are presented as weak positive, positive and negative. The presence of blood and saliva cannot affect the test, except to prevent proper visualization of the color when reading the result[6].

Starting from the fact that the main causal factor for periodontal diseases is periopathogenic microorganisms present in dental plaque, their elimination is the primary goal of periodontal therapy.

There are conservative and surgical methods of choice in the treatment of periodontal disease, which are aimed at effective removal of the dental plaque. Although conservative treatment in periodontology (ultrasound instrumentation and treatment of the root surface of the teeth) is considered the gold standard, it does not always give the required effect. This is due to variations in tooth anatomy, the depth of periodontal pockets, and also the ability of some specific bacteria to invade tissues. These factors impose the necessity of using various antimicrobial agents, prescribed systemically or locally, as a complement to conservative treatment.

The advantage above all is given to the locally applied antimicrobial agents, for the purpose of maintaining a higher and longer-term concentration of the medication in the affected region, its reduced or excluded systemic absorption and, most importantly, reducing the possibility of the creation of resistant strains of bacteria.

Over the years, various antimicrobial agents have been used for subgingival application, which have shown significant results in the treatment of periodontal diseases.

Recently, the benefits of using ozone as a supplement to the conservative therapy of periodontal disease have been mentioned more often[6]. The word "ozone" was used by Schonbein in 1840 and is derived from the Greek word "ozein" which literally means "smell; it smells"[7].

Ozone is a molecule with three oxygen atoms and is an unstable gas with a half-life of 40 minutes at a temperature of 20 degrees. Its applicability in medicine and dentistry is indicated in the treatment of over 260 different pathologies. Ozone therapies have been shown to be more beneficial than existing minimally invasive treatment modalities in dentistry.

There are three fundamental forms of application of ozone in the oral cavity: ozonated water, ozonated olive oil and ozone in the form of gas. Ozonated water and ozonized olive oil are an ideal system for the transfer of ozone to the tissues, because they have the capacity to trap and then release oxygen molecules, which is how they achieve their antibacterial effect against the periodontopathogenic species present[8].

Aim

Monitoring the therapeutic effects of ozonated olive oil on periopathogenic microorganisms from the red microbial complex (*T. denticola, T. forsythia, P. gingivalis*), as well as its effects on the periodontal status, by monitoring the clinical periodontal parameters.

Material and methods

To realize the set goal, 60 patients with clinically manifested periodontal disease were analyzed.

The diagnosis was made on the basis of a clinical periodontal examination and panoramic x-rays.

The study was conducted following the principles of the World Medical Association Declaration of Helsinki, with the approval of the Ethics Committee of the Faculty of Dentistry.

All respondents included in the study gave written consent to participate in the realization of the research.

Criteria for the inclusion of participants in the study were as follows:

- patients with diagnosed chronic periodontal disease;
- patients who did not have systemic diseases as an additional risk, a factor that has an impact on the course of periodontal disease;
- patients who did not receive antibiotic therapy and did not use local antiseptics in the last three months.
- Patients were divided into two groups:
- First group, 30 subjects, who underwent conservative periodontal treatment (ultrasound instrumentation and treatment of the periodontal pocket).
- The second group, 30 subjects in whom the conservative periodontal treatment was supplemented with the application of ozone therapy in the form of ozonated olive oil.

Ozonated olive oil (Ozone Gold-Germany, GmbH, 80mg) was applied subgingivally, in the region around the periodontal pocket, on three occasions (one, two and three weeks after the initial treatment) with an appropriately sized needle. After the application, patients abstained from consuming food and drinks for a period of 30 minutes.

For monitoring the effects of applying ozone therapy, as an additional therapeutic modality to conservative periodontal treatment, the periodontal status was recorded through the periodontal index parameters:

DPI (Dental Plaque Index, Silness-Loe)[9];

IGI (Index of gingival inflammation, Loe-Silness)[10];

GBI (Gingival Bleeding Index, Ainamo and Bay)[11];

CAL (Clinical Attachment Loss)[12].

These index parameters were taken before the start of the conservative treatment and one month after conservative therapy combined with ozone therapy.

Examinations were carried out at the Clinic for Diseases of the Mouth and Periodontics at PHI University Dental Clinic "St. Pantelejmon", Faculty of Dentistry in Skopje.

Microbiological tests to detect the presence or absence of the three anaerobic periopathogenic bacterial species from the red complex (*T. denticola, T. forsythia, P. gingivalis*) were performed using the BANA test.

For the realization of this test, a swab was taken from the two deepest active periodontal pockets. With the help of a subgingival curette, the swab was applied to two separate places on the designed strip for BANA test.

Using the BANA processor, the results were processed and read based on the scale described by Loesche *et al.* (1990), weakly positive, positive and negative[5].

The microbiological tests were repeated in all subjects after one month.

BANA - the test was performed in the Center for scientific-research work with laboratories within the University Dental Clinic "St. Panteleimon", Faculty of Dentistry in Skopje.

Statistical processing of the results and analysis

Evaluation of the results from the conducted clinical and microbiological examinations were statistically processed, using the SPSS software package, version 20.0 for Windows (SPSS, Chicago, IL, USA).

Results

Sixty subjects participated in the study; patients with clinically manifested periodontal disease, aged 45 to 70 years and an average age of 57.0 ± 7.4 years. There were 27 (45%) male and 33 (55%) female patients (Table 1).

Table 1. Gender structure of patients

	Variable	n (%)
Gender	male	27(45)
	female	33(55)
Age(years)	(mean±SD) (min-max)	(57.0±7.4) (45-70)

Patients were divided into 2 groups:

Group 1: 30 patients who underwent conservative periodontal treatment (CT).

Group 2: 30 patients, in whom the conservative periodontal treatment was supplemented with ozonized oil (CT+OO) (Figure 1).

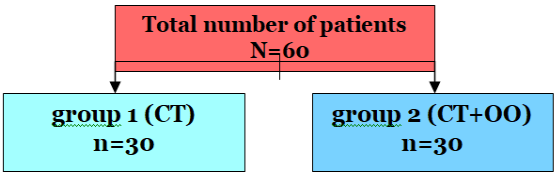

Fig. 1. Graphical display of groups

Table 2. Dental plaque index in the group with CT

DPI Dental plaque index	CT Admission n (%)	Check-up n (%)	p value
0 - No plaque is in the area adjacent to the gingiva		4 (13.33)	
1- There is a plaque in the form of a thin film on the gingival margin	3(10)	26 (86.67)	
2 - There is a visible plaque in the gingival pocket and gingival margin	22(73.33)		Z=4.8 ***p=0.000002
3 - There is a dense plaque in the gingival pocket and on the gingival margin	5 (16.67)		

Z (Wilcoxon Matched Pairs Test); ***p<0.0001

In the group with applied conservative treatment, all patients on admission had dental plaque, with the majority having a moderate amount of dental plaque, DPI= 2.

At the control examination, 26(86.67%) patients had dental plaque in a small amount, DPI=1 (Table 2).

For p=0.000002, a significant decrease in DPI of the control versus the admission value was confirmed.

All patients treated conservatively in combination with ozone oil had dental plaque on admission, mostly in moderate amount, DPI=2.

At the control, 23(76.67%) had dental plaque in a small amount, DPI=1.

The statistical analysis confirmed a significant reduction of DPI at the control examination (p=0.000002) in patients who received conservative treatment supplemented with ozone oil (Table 3).

Table 3. Dental plaque index in the group with CT + OO

DPI **Dental plaque index**	**CT+OO** **Admission** **n (%)**	**Check-up** **n (%)**	**p value**
0 - No plaque is in the area adjacent to the gingiva		7(23.33)	
1- There is a plaque in the form of a thin film on the gingival margin	6(20)	23(76.67)	
2 - There is a visible plaque in the gingival pocket and gingival margin	18(60)		Z=4.8 ***p=0.000002
3 - There is a dense plaque in the gingival pocket and on the gingival margin	6(20)		

Z (Wilcoxon Matched Pairs Test); ***p<0.0001

On admission, conservatively treated patients mostly had moderate gingival inflammation, IGI=2.

At the follow-up examination, after one month of conservative periodontal treatment, 14(46.67%) patients had mild inflammation of the gingiva, IGI=1, with a statistical significance (p=0.000003) (Table 4).

Table 4. Index of gingival inflammation in the group with CT

IGI Index of gingival inflammation	**CT** **Admission** **n (%)**	**Check-up** **n (%)**	**p value**
Healthy gums		16(53.33)	
Mild inflammation	5(16.67)	14(46.67)	Z=4.7 ***p=0.000003
Moderate inflammation	25(83.33)		

Z (Wilcoxon Matched Pairs Test); ***p<0.0001

On admission, patients with moderate inflammation in the CT+OO group dominated, IGI =2.

At the control examination, for p=0.000002, a significant decrease in the IGI value was confirmed, i.e., 60% of patients were without inflammation, and in the remaining 40%, weak gingival inflammation was registered, IGI=1 (Table 5).

In the group with applied conservative treatment, all patients on admission had gingival bleeding, 16.67% had GBI=1, 70% had GBI=2, and 13.33% had GBI=3.

Table 5. Index of gingival inflammation in the group with CT+OO

	КТ + ОМ		
IGI Index of gingival inflammation	**Admission n (%)**	**Check-up n (%)**	**p value**
Healthy gums		18 (60%)	Z=4.8 ***p=0.000002
Mild inflammation	7 (23.33)	12 (40)	
Moderate inflammation	23 (76.67)		

Z (Wilcoxon Matched Pairs Test); ***p<0.0001

At the control examination, after one month of conservative periodontal treatment, the statistical analysis confirmed a significant decrease in the GBI value. Specifically, 11 (36.67%) patients had gingival bleeding with low intensity, GBI=1, while 63.33% had no gingival bleeding, GBI=0 (Table 6).

Table 6. Gingival bleeding index in the group with CT

	КТ		
GBI Gingival bleeding index	**Admission n (%)**	**Check-up n (%)**	**p value**
No bleeding		19(63.33)	
Mild bleeding	5(16.67%)	11(36.67)	Z=4.7
Moderate bleeding	21(70%)		***p=0.000003
Severe bleeding	4(13.33)		

Z (Wilcoxon Matched Pairs Test); ***p<0.0001

Twenty-two or 73.33% of patients in the group treated with CT+OO on admission most often had moderate gingival bleeding, GBI =2, followed by an equal representation of patients (13.33%), with mild and severe gingival bleeding.

No patients with gingival bleeding were registered at the control examination of this group).

The statistical analysis confirmed a significant decrease in GBI value after one month of combined periodontal treatment (p=0.000000) (Table 7).

Table 7. Gingival bleeding index in the group with CT+OO

	CT+OO		
GBI Gingival bleeding index	**Admission n (%)**	**Check-up n (%)**	**p value**
No bleeding		30(100)	***p=0.000000
Mild bleeding	4(13.33)		
Moderate bleeding	22(73.33)		
Severe bleeding	4(13.33)		

Z (Wilcoxon Matched Pairs Test); ***p<0.0001

On admission, in the group treated only conservatively, in 53.33% of patients, CAL most often had a value of 5, while after one month of treatment, in 43.33% of patients, the

Table 8. Clinical attachment loss in the group with CT

	CT+OO		
CAL Clinical attachment loss	**Admission n (%)**	**Check-up n (%)**	**p value**
3		4(13.33)	Z=2.5 *p=0.0117
4	9(30)	13(43.33)	
5	16(53.33)	8(26.67)	

6	5(16.67)	5(16.67)

Z (Wilcoxon Matched Pairs Test); *p<0.05

most frequently registered value of CAL was 4 (Table 8).

The statistical analysis confirmed significantly lower CAL values at the control examination in patients treated only conservatively (p=0.0117) (Table 8).

Patients treated with combined therapy, conservatively and with ozonized olive oil on admission most often had a CAL value of 5.

At the control examination, for p=0.0077, a significant decrease in CAL values was confirmed, the same percentage of respondents (53.33%) showed a decrease in the CAL value to 4 (Table 9).

Table 9. Clinical attachment loss in the group with CT+OO

CAL Clinical attachment loss	**CT+OO Admission n (%)**	**Check-up n (%)**	**p value**
3		4(13.33)	
4	11(36.67)	16(53.33)	Z=2.7
5	16(53.33)	7(23.33)	p=0.0077
6	3(10)	3(10)	

Z (Wilcoxon Matched Pairs Test); **p<0.01

On admission, all patients had a positive BANA test (Table 10).

Table 10. BANA / admission by groups

BANA admission	**n**	**Groups group 1 n (%)**	**group 2 n (%)**
positive	30	30(100)	30(100)
negative	30	30(100)	30(100)

At the control examination, 10 patients (33.33%) from the conservatively treated group had a positive BANA test (Table 11).

Table 11. BANA / check-up by groups

BANA checkup	**Groups**			**P value**
	n	**Group 1 n (%)**	**Group 2 n (%)**	
positive	10	10(33.33)	0	X^2=12.0
negative	50	20(66.67)	30(100)	***p=0.00053
Group 1-CT (conservative treatment), Group 2-CT+OO (conservative treatment+ozonized olive oil), X^2 (Pearson Chi-square test)				

All patients treated conservatively combined with ozonized olive oil, had a negative BANA test at the follow-up examination.

For p=0.0005, a statistically significant difference was confirmed in the distribution of patients with a positive and negative control BANA test, depending on the type of periodontal treatment.

The combined treatment showed a significantly greater success than the conservative one, in terms of the presence of the three anaerobic periodontopathogenic bacterial species (*T. denticola, T. forsythia, P. gingivalis*).

Discussion

The start and progression of periodontal disease is caused by various bacterial accumulations in the subgingival pockets. Early detection of periodontal disease is actually very difficult, but at the same time very important for starting appropriate treatment that would prevent further progression of the disease itself. The elimination of pathogenic subgingival microflora can be achieved mechanically. However, the effectiveness of this method is limited by numerous factors such as: the concave surfaces of the teeth, the edges of the restorations and the inaccessibility of the periodontal pockets.

The chronic course taken by the disease requires additional treatment to maintain the results of the initial therapy[13].

The use of ozone therapy has a long history of research and clinical trials in humans[8].

Most of the studies related to the use of ozone in the treatment of periodontal disease have shown positive results[14-17].

In the study of Ana Maria Ramirez-Pena *et al*., 2022, it was concluded that ozone in the form of medical gas was effective in the treatment of generalized periodontal disease and as such had a great benefit due to the fact that this therapy is minimally invasive, economically viable, painless and easily accepted as a therapeutic model by patients[14]. Ozone reacts with various chemical compounds in two distinct and coexisting ways, one involving direct reactions of molecular ozone and the other a reaction mediated by free radicals[18]. Both mechanisms of action represent ways in which ozone participates in the destruction of bacteria. There are several ways of delivering ozone. One is when ozone dissolves in water. But in doing so, it becomes very unstable and breaks down quickly through a complex series of chain reactions and therefore cannot be stored. In contrast, when dissolved in an oil base, it has a lifespan that can be measured in years. In this way, the ozone chemically reacts with the oil to form long complex molecules[19,20].

Gupta G. *et al*., 2012, investigating the clinical applicability of ozone indicated that ozone can be used in the treatment of periodontal diseases.

Namely, with the use of ozonized water (4 mg/l), that is, with rinsing of the oral tissues, there is an inhibition in the formation of dental plaque and a reduction in the number of subgingival pathogens, Gram-positive and Gram-negative microorganisms. Gram-negative bacteria such as *P. endodontalis* and *P. gingivalis* showed greater sensitivity to ozonated water than Gram-positive oral streptococci and *C. albicans*.

They also observed that ozonated water inhibited experimental dental plaque *in vitro*[21].

In the study by Sorokina and Lukinych[22], using subgingival application of ozonized water in periodontal pockets, in combination with professional oral hygiene measures, reduced formation of dental plaque was registered, as well as potentiated anti-inflammatory effects on periodontal tissues.

In a randomized study by A. Piva *et al*., 2020, including 10 patients, 5 men and 5 women, aged 42-73 years, analysis was conducted on the benefits of using ozonized water (Ozonline®) in the treatment of periodontal disease. For this purpose, in all patients, the upper and lower right quadrants in the mouth were treated only with ultrasound instrumentation, while the upper and lower left quadrants were treated with ultrasound instrumentation and ozonized water (Ozonline®). In each of the patients, 10 microbiological samples from the upper left quadrants and 10 samples from the upper right quadrants were taken at the sites with localized chronic periodontal disease at the very beginning of the therapy and on the 7th day of the therapy. In places where ozonized water (Ozonline®) was used in the therapy itself, a significant reduction in the number of microorganisms was observed. *T. forsythia* and *T. denticola* were completely removed, while the total number of bacteria and *Fusobacterium nucleatum* were reduced by 38% and 55% in the right quadrants[23].

Patel *et al.*, 2012[24], confirmed that conservative periodontal treatment supplemented with the use of ozonated olive oil gel resulted in a significant improvement (P<0.001) of clinical and microbiological parameters, compared to control groups without any documented side effects.

The study by Marco Colombo *et al*., 2021, about the use of ozonized gel (GeliO3) in the management of non-surgical treatment of periodontal disease, although without a greater effect compared to conservative treatment supplemented with the use of chlorhexidine, showed better results by using ozone due to the fact that chlorhexidine had greater cytotoxic effects[25].

In our study, we gave preference to ozonated olive oil over ozonated water because it has been confirmed that in this way it allows its longer stay in the oral cavity, adequate penetration, high efficiency and acceptability, which is also due to the action of Omega 3 fatty acids in olive oil that has potential benefits as a modulatory agent in the adjuvant treatment of periodontal disease[26,27].

The results of our study indicated that in both groups of subjects there was an improvement in the values for all examined clinical parameters (DPI, IGI, GBI, CAL), with a statistically significant improvement in the values in the group where ozonated olive oil with a concentration of 80 mg (Ozone Gold-Germany, GmbH) was used as an adjunct to the conservative treatment of periodontal disease.

For p=0.0005, a statistically significant difference was confirmed in the distribution of patients with a positive and negative control BANA test, depending on the type of periodontal treatment.

Microbiological tests for the rapid detection of the presence or absence of the three anaerobic periopathogenic bacterial species from the red complex (*T. denticola, T. forsythia, P. gingivalis*), indicated that all patients treated with a combined therapy, i.e., conservatively and with ozonized olive oil, had a negative BANA test at the control examination.

The combined treatment showed a significantly greater success than the conservative one in terms of the presence of the three anaerobic periopathogenic bacterial species (*T. denticola, T. forsythia, P. gingivalis*).

Our results are in agreement with the study conducted by Shoukheba *et al*., which investigated the effects of using ozonated olive oil as an adjunct to conservative treatment in the therapy of aggressive periodontitis. The results revealed that in the group in which, in addition to the conservative treatment, ozonized olive oil was used, there were improvements in all clinical parameters, maintaining the results for a period of 6 months (p<0.005), except for the occurrence of bleeding during probing, which maintained the results only 3 months.

In comparison, the group that was not treated with ozone, the improvement results were maintained for only 1 month for all parameters, except for the plaque index and gingival index parameters, which were maintained up to 3 months after therapy. From a bacteriological point of view, the results showed a statistical significance for the reduction of DNA copies of *P. gingivalis* in 1 month and in 3 months in the group treated with ozone [28]. In a study by Eick S. *et al*.., about the effects of ozone on periopathogenic species, it was concluded that ozone had the potential to be used as an adjunct to mechanical conservative therapy in patients with periodontal disease. It was also confirmed that it had a strong antibacterial effect against the present periopathogenic species, but that its bactericidal effect was reduced in the presence of serum[29].

Conclusion

Considering the limitation of this study regarding the short period of observation, it can be concluded that the local application of ozonized olive oil in addition to conservative

periodontal treatment can serve as a potential atraumatic, promising antimicrobial agent in the non-surgical treatment of periodontal disease. However, additional long-term studies are necessary for an adequate assessment of the concentration of ozone that would be effective against periopathogenic microorganisms.

Conflict of interest statement. None declared.

References:

1. Locker D, Slade GD, Murray H. Epidemiology of periodontal disease among older adults: A review. *Periodontol 2000* 1998; 16: 16-33. doi: 10.1111/j.1600-0757.1998.tb00113.x.
2. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 2005; 366(9499): 1809-1820. doi: 10.1016/S0140-6736(05)67728-8.
3. Savage A, Eaton KA, Moles DR, Needleman I. A systematic review of definitions of periodontitis and methods that have been used to identify this disease. *J Clin Periodontol* 2009; 36(6): 458-467. doi: 10.1111/j.1600-051X.2009.01408.x.
4. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. *J Clin Periodontol* 1998; 25(2): 134-144. doi: 10.1111/j.1600-051x.1998.tb02419.x.
5. Loesche WJ, Bretz WA, Kerschensteiner D, Stoll J, Socransky SS, Hujoel P, *et al*. Development of a diagnostic test for anaerobic periodontal infections based on plaque hydrolysis of benzoyl-DL-arginine-naphthylamide. *J Clin Microbiol* 1990; 28(7): 1551-1559. doi: 10.1128/jcm.28.7.1551-1559.1990.
6. Nambiar S, Malothu S, Karmakar S, Varkey A, Chandra D, Chava VK. Comparison of Ozonated Olive Oil and Chlorhexidine Gel as an Adjunct to Nonsurgical Periodontal Therapy for the Treatment of Chronic Periodontitis: A Randomized Controlled Clinical Trial. *J Pharm Bioallied Sci* 2022; 14(Suppl 1): S94-S98. doi: 10.4103/jpbs.jpbs_565_21.
7. Deepthi R, Bilichodmath S. Ozone Therapy in Periodontics: A Meta-analysis. *Contemp Clin Dent* 2020; 11(2): 108-115. doi: 10.4103/ccd.ccd_79_19.
8. Saini R. Ozone therapy in dentistry: A strategic review. *J Nat Sci Biol Med* 2011; 2(2): 151-153. doi: 10.4103/0976-9668.92318.
9. Silness J, Loe H. Periodontal disease in pregnancy. 2. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964; 22: 121-135. doi: 10.3109/00016356408993968.
10. Loe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity. *Acta Odontol Scand* 1963; 21: 533-551. doi: 10.3109/00016356309011240.
11. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J* 1975; 25(4): 229-235. PMID: 1058834.
12. Highfield, J. Diagnosis and classification of periodontal disease. *Australian Dental Journal* 2009; 54(Suppl 1): S11-S26. doi: 10.1111/j.1834-7819.2009.01140.x.
13. Graetz C, Ehrenthal JC, Kowalski R, Cyris M, Geiken A, Dörfer CE. Periodontal maintenance: individual patient responses and discontinuations. *BMC Oral Health* 2022; 22(1): 610. doi: 10.1186/s12903-022-02655-8.
14. Ramirez-Peña AM, Sánchez-Pérez A, Campos-Aranda M, Hidalgo-Tallón FJ. Ozone in Patients with Periodontitis: A Clinical and Microbiological Study. *J Clin Med* 2022; 11(10): 2946. doi: 10.3390/jcm11102946.
15. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. *J Endod* 2004; 30(11): 778-781. doi: 10.1097/00004770-200411000-00007.

16. Ramzy MI, Gomaa HE, Mostafa MI, Zaki BM. Management of aggressive periodontitis using ozonized water. *Egypt Med JNR C* 2005; 6: 229-245.
17. Huth KC, Quirling M, Lenzke S, Paschos E, Kamereck K, Brand K, *et al.* Effectiveness of ozone against periodontal pathogenic microorganisms. *Eur J Oral Sci* 2011; 119(3): 204-210. doi: 10.1111/j.1600-0722.2011.00825.x.
18. Staehelin J, Hoigne J. Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. *Environ Sci Technol* 1985; 19(12): 1206-1213. doi: 10.1021/es00142a012.
19. Hoigne J, Bader H. The role of hydroxyl radical reactions in ozonation processes in aqueous solutions. *Water Res* 1976; 10(5): 377-386. doi: 10.1016/0043-1354(76)90055-5.
20. Shin WT, Mirmiran A, Yiacoumi S, Tsouris C. Ozonation using micro bubbles formed by electric fields. *Separat Purif Technol* 1999; 15(3): 271-282. doi: 10.1016/S1383-5866(98)00107-5.
21. Gupta G, Mansi B. Ozone therapy in periodontics. *J Med Life* 2012; 5(1): 59-67. PMID: 22574088.
22. Sorokina S, Lukinych L. Ozone therapy as a part of a complex treatment of a paradontium disease. *The medical academy of Nizhni Novgorod, RussiaSource: 2nd International Symposium on Ozone Applications, Havana, Cuba* 1997; pp. 24-26.
23. Piva A, Avantaggiato P, Candotto V, Pellati A, Moreo G. The use of ozone therapy for treatment of periodontal disease: a split-mouth, randomized, controlled clinical trial. *J Biol Regul Homeost Agents* 2020; 34(3 Suppl 1): 91-98. PMID: 32618165.
24. Patel PV, 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): 381-398. PMID: 22976566.
25. Colombo M, Gallo S, Garofoli A, Poggio C, Arciola CR, Scribante A. Ozone Gel in Chronic Periodontal Disease: A Randomized Clinical Trial on the Anti-Inflammatory Effects of Ozone Application. *Biology* 2021; 10(7): 625. doi: 10.3390/biology10070625.
26. Jain N, Jain GK, Javed S, Iqbal Z, Talegaonkar S, Ahmad FJ, *et al*. Recent approaches for treatment of periodontitis. *Drug Discov Today* 2008; 13(21-22): 932-943. doi: 10.1016/j.drudis.2008.07.010.
27. Rosentein ED, Kushner LJ, Kramer N, Kazandjian G. Pilot study of dietary fatty acid supplementation in the treatment of adult periodontitis. *Prostaglandins Leukot Essent Fatty Acids* 2003; 68(3): 213e8. doi: 10.1016/S0952-3278(02)00272-7.
28. Shoukheba MYM, Ali ShA. The effects of subgingival application of ozonated olive oil gel in patient with localized aggressive periodontitis. A clinical and bacteriological study. Tanta Dental Journal. 2014; 11(1): 63-73. doi: 10.1016/j.tdj.2014.04.001.
29. Eick S, Tigan M, Sculean A. Effect of ozone on periodontopathogenic species - an in vitro study. *Clin Oral Invest* 2012; 16(2): 537-544. doi: 10.1007/s00784-011-0515-1.