Original Article / Özgün Araştırma

Meandros Med Dent J
doi:10.4274/meandros.galenos.2021.90532
Meandros Med Dent J 2023;24(2):119-124

119

Effects of Ozone Threapy as an Adjunct to Non-Surgical Periodontal Treatment on Clinical Periodontal Parameters and Inflammatory Markers in Periodontitis Patients

Periodontitisli Bireylerde Cerrahi Olmayan Periodontal Tedaviye Ek Olarak Uygulanan Ozonun Klinik Periodontal Parametrelere ve Enflamatuvar Belirteç Seviyelerine Etkileri

Sema Nur Sevinç Gül[1], Alparslan Dilsiz[1], Nurinnisa Özturk[2], Mehmet Ali Gül[3]

[1]Atatürk University Faculty of Dentistry, Department of Periodontology, Erzurum, Turkey
[2]Atatürk University Faculty of Medicine, Department of Medical Biochemistry, Erzurum, Turkey
[3]Amasya University Faculty of Medicine, Department of Medical Biochemistry, Amasya, Turkey

Abstract

Objective: Ozone therapy (OT) is known to eliminate pathogens in the periodontal pocket and accelerate wound healing. This study aimed to investigate the clinical and biochemical efficacy of OT as adjunctive scaling and root planning (SRP) in the treatment of periodontitis.

Materials and Methods: Fifty systemically healthy individuals with periodontitis were included in this study. Gingival crevicular fluid (GCF) samples were taken from the patients before and 8 weeks after starting the treatment, and gingival index (GI), plaque index (PI), bleeding on probing (BOP), pocket depth (PD), and clinical attachment level (CAL) were obtained from all subjects. Non-surgical periodontal treatment was applied to the test and control groups, and gaseous ozone treatment was applied in addition to the test group. TNF-α, IL-1β, IL-6, IL-10, and MMP-9 values in GCF samples before and after treatment were determined by the ELISA method and evaluated statistically.

Results: Both groups presented significantly decreased clinical periodontal parameters at 8-week from baseline ($p<0.05$). Clinical periodontal parameters were found to be significantly lower in the test group than In the control group, there was a decrease in TNF-α levels after non-surgical periodontal treatment ($p<0.05$). Both groups demonstrated significantly decreased IL-1β, IL-6, IL-10, and MMP-9 levels at 8-week ($p<0.05$). The test group showed a significant decrease in MMP-9 levels compared with the control group at 8-week ($p<0.05$).

Conclusion: As a result of the close relationship observed between clinical periodontal measurements and biochemical parameters, it was observed that clinical periodontal healing affects biochemical parameters, and in addition to non-surgical periodontal treatment, ozone treatment improves clinical periodontal measurements and affects biochemical parameters by reducing the GCF volume.

Keywords: Periodontitis, cytokines, inflammation, matrix metalloproteinases, ozone, periodontal debridement

Öz

Amaç: Medikal ozon tedavisinin, periodontal cepteki patojenleri elimine ettiği ve yara iyileşmesini hızlandırdığı bilinmektedir. Bu çalışmanın amacı, periodontal hastalıklı bireylerde ozon tedavisinin klinik ve biyokimyasal olarak etkilerinin değerlendirilmesidir.

Gereç ve Yöntemler: Bu çalışmaya sistemik olarak sağlıklı ve periodontitisli 50 birey dahil edildi. Tedaviye başlamadan önce ve 8 hafta sonra hastalardan dişeti oluğu sıvısı (DOS) örnekleri alınmış, tüm bireylerden gingival indeks, plak indeksi, sondalamada kanama, sondalamada cep derinliği ve klinik ataçman seviyesini içeren klinik periodontal ölçümler elde edilmiştir. Test ve kontrol grubuna cerrahi olmayan periodontal tedavi yapılmış, test grubuna ek olarak gaz ozon tedavisi uygulanmıştır. Tedavi öncesi ve sonrası, DOS örneklerinde Tumor necrosis factor-α (TNF-α), IL-1β, IL-6, IL-10 ve MMP-9 değerleri ELİSA metoduyla saptanmış ve istatistiksel olarak değerlendirilmiştir.

Address for Correspondence/Yazışma Adresi: Sema Nur Sevinç Gül Asst. Prof., Atatürk University Faculty of Dentistry, Department of Periodontology, Erzurum, Turkey
Phone: +90 507 991 00 07 **E-mail:** semanursevinc@gmail.com
ORCID ID: orcid.org/0000-0003-0699-917X

Received/Geliş Tarihi: 22.10.2021
Accepted/Kabul Tarihi: 10.12.2021

Bulgular: Hem test hem de kontrol grubunda cerrahi olmayan periodontal tedavi sonrası klinik periodontal ölçümlerde başlangıç değerlerine göre azalma saptanmıştır ($p<0,05$). Test grubunda, kontrol grubuna göre, klinik periodontal ölçümlerde azalma tespit edilmiştir ($p<0,05$). Her iki grupta da DOS IL-1β, IL-6, IL-10 ve MMP-9 seviyelerinde başlangıç değerlerine göre, cerrahi olmayan periodontal tedavi sonrası azalma gözlenmiştir ($p<0,05$). Kontrol grubunda DOS TNF-α seviyesinde başlangıç değerlerine göre, cerrahi olmayan periodontal tedavi sonrasında azalma saptanmıştır ($p<0,05$). Test grubunda kontrol grubuna göre, DOS MMP-9 seviyesinde azalma gözlenmiştir ($p<0,05$).

Sonuç: Klinik periodontal ölçümler ve biyokimyasal parametreler arasında gözlenen yakın ilişki, klinik periodontal iyileşmenin biyokimyasal parametreleri etkilediği, cerrahi olmayan periodontal tedaviye ek olarak ozon tedavisinin DOS hacmini azaltarak klinik periodontal ölçümleri iyileştirdiği ve biyokimyasal parametrelere etki ettiği de gözlenmiştir.

Anahtar Kelimeler: Periodontitis, sitokinler, enflamasyon, matriks metalloproteinaz, ozon, periodontal debridman

Introduction

Periodontal disease stems from the reaction of the host defense mechanisms with the plaque microorganisms, which destroy the supporting tissues of the teeth (1). The basis of non-surgical periodontal therapy is the mechanical elimination of microbial plaque and calculus by scaling and root (SRP). However, SRP alone may be insufficient to destroy the pathogenic bacteria, due to their location in the gingival and dental tissues or in other sites that are not easily accessed by periodontal instruments (2). Local or systemic antibiotics and antiseptics are suggested to improve the results of periodontal therapy (3).

Ozone therapy (OT), widely used in dentistry, has been proven *in vitro* and *in vivo* studies to be effective in the removal of pathogenic microorganisms in periodontal disease (4,5). Medical ozone has very high oxidation power, it is recognized as "active oxygen" in the medical field (6). OT also stimulates the immune system by releasing cytokines such as interferon and interleukin (IL) by leukocytes (7,8). Studies are stating that OT as an adjunct to SRP contributes to the improvement in clinical parameters in patients with periodontitis (9,10). This study was aimed to evaluate the clinical and biochemical efficacy of gas OT as an adjunctive to SRP in patients with periodontitis.

Materials and Methods

This study was a randomized-controlled, double-blind clinical trial, conducted between October 2014 and October 2015 in a single centre, and each individual was followed up for 8 weeks. The study protocol was confirmed by the Ethics Committee of Atatürk University, Erzurum, Turkey (decision code: 24/2014, date: 13.10.2014), and managed according to the principles outlined in the Declaration of Helsinki on experimentation involving human individuals. The signed consent form was obtained from all participants.

Selection of Patient

A total of 50 healthy, non-smoking patients with periodontitis (19 women and 31 men) between 31 to 57 were selected. All patients presented stage III periodontitis, based on clinical parameters [plaque index (PI), gingival index (GI), probing pocket depth (PD), clinical attachment level (CAL)]. Patients of each group had a minimum of 20 teeth and at least two pocket site with a probing depth 5 mm or more in each quadrant. Patients with periodontitis were classified according to the 1999 classification at the time of treatment, but were revised according to the 2017 periodontal disease classification defined by the European Federation of Periodontology and the American Academy of Periodontology (11) classification criteria. Patients with stage III periodontitis according to current classification were included in the study. All individuals were generally healthy, non-smoking, and none had undergone periodontal therapy and/or antibiotic therapy in the past 6 months. Pregnant or breastfeeding women and tobacco users were excluded from the study. Depending on the treatment plan, the patients were divided into 2 groups.

Control group: This group included 25 patients with stage III periodontitis (9 women and 16 men) aged between 31 to 57 who were applied SRP.

Test group: Test group consisted of 25 patients stage III periodontitis (10 women and 15 men), aged between 33 to 55, who were applied OT as an adjunctive to SRP.

Power Analysis

The sample size was determined by showing regard to α=0.05 and power (1-β)=85%. Because of the variability value (σ=SD), a 0.05 mm change in the clinical attachment gain was used. The clinically allowable mean value (δ) was taken as 0.5 mm. Relying on these data, the minimum number of patients required for this study was computed as 22 for each group

Study Design

The study design summerized Figure 1. Before treatment was initiated, the randomized determination of the test or control groups was performed using a coin toss. Two calibrated rearchers (A.D., S.S.) conducted the study. Only one researcher (S.S.) conducting clinical evaluation was blinded to the treatment undergone by patients. The treatment groups were encoded, therefore only the operator (A.D.) was informed of the protocol and the examiner maintained blinded during the study. The clinical examination in both groups was performed two times: baseline and eight weeks after treatment. The parameters were calculated by

a periodontal probe (Williams; Hu-Friedy). The following parameters was assesed: PI, GI, PD, CAL were measured at six sites of each tooth (mesialbuccal, buccal, distalbuccal, mesiolingual, lingual and distolingual). Only periodontal pockets depth of 5 mm and above were included in the assessment. Before SRP, patients were given oral hygiene training including toothbrush and dental floss and/or interdental brushes were called for control 1 week later and gingival crevicular fluid (GCF) samples were taken.

Examiner Calibration

The investigator (S.S.) conducting clinical evaluation was calibrated for intra-examination repeatability before the study initiation. Five patients with 10 defects were included as this reason. Duplicate parameters of PD were gathered with a period of 48 hours among the first and the second record. Standardization was approved whereas parameters at baseline and at 48 h were within a milimeter (mm) at ≥90% of the time.

Treatment Procedure

The study was performed in the Department of Periodontics, Faculty of Dentistry, Ataturk University, Erzurum, Turkey.

Test Group: Each of the patients received SRP. Mechanical debridement was applied with ultrasonic piezoelectric scalers (EMS, Mini-Piezon) and hand instruments (Gracey curettes, Hu-Friedy). Following SRP in the test group, gas ozone application into the periodontal pockets was carried out by the capillary tip (KPX) for 60 sec at 26 µg power (16,600 ppm at 60% power) accordance to the parameters specified by the manufacturer company (Ozonytron XP, MYMED Gmb H). The application of each tooth are made in equal time careffully. Gas ozon was applied two times for three consecutive days. Aspiration was performed to prevent ozone inhalation during application.

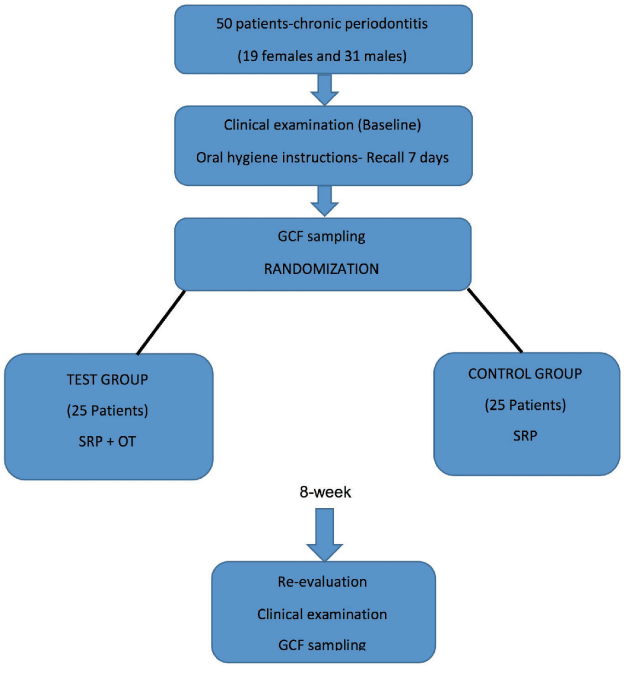

Figure 1. Study desing

GCF: Gingival crevicular fluid, SRP: Saling and Root Planing, OT: Ozone therapy

Control Group: Only SRP was applied.

Sample Collection

GCF samples were collected from four affected teeth using paper strips (PeriopaperTM, Oraflow Inc). The procedure was continued for 30 seconds at the gingival sulcus for each tooth. The sample volumes were measured with a calibrated specific device (Periotron 8000®, Oraflow Inc). The strips stored at -80 °C prior to use. GCF sampling and clinical periodontal parameters were obtained before SRP for both groups and after 8 weeks period.

Biochemical Analysis

Biochemical analyses were evaluated with the "Enzyme Linked-Immuno-Sorbent Assay" (ELISA) method using a diagnostic tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-10 (IL-10) (eBioscience, Bender Medsystems) and matrix metalloproteinases-9 (MMP-9) (Quantikine®, R&D Systems) ELISA kit.

Statistical Analysis

Statistical analyses was performed by using SPSS 20.0 program.

The results were described as the mean ± standard deviation (SD). The normal distribution suitability of the parameters was determined by the Kolmogorov-Smirnov test. Because all parameters were normalized, pre-and post-treatment values, which were assessed by t-test (paired samples t-test) for dependent samples, are used to compare the mean of numerical data from two dependent groups. The student t-test for dependent samples were done with differences over time in the related groups. Pearson correlation analysis was applied with regard to correlation analysis. The value of $p<0.05$ was taken into account to be statistically significant.

Results

The test group consisting of 10 females and 15 males with a mean age of 42.16±6.32 with periodontitis was carried out on the control group consisting of 9 females and 16 males with a mean age of 40.16±8.04. There was no significant difference between the groups in terms of age and gender distribution ($p>0.05$).

The mean and SDs of the clinical periodontal parameters and GCF volumes obtained from baseline and after 8-week are shown in Table 1. There was no statistically significant difference between groups baseline clinical periodontal parameters and the GCF volume ($p>0.05$).

There was no statistically significant difference between the test and control groups in the GCF volume after 8-week

(p>0.05), but there was a statistically significant reduction in the clinical periodontal parameters (p<0.05).

In the test and control groups, a statistically significant decrease in all clinical periodontal parameters and GCF volumes after baseline and after 8-week were observed (p<0.05).

The mean values of biochemical markers between baseline and after 8-week and statistical comparison of these values between the test and control groups are given in Table 2.

There was no statistically significant difference between the mean values of baseline levels of biochemical marker between the test and control groups (p>0.05)

In the test and control group, the baseline levels of IL-1β, IL-6, IL-10 and MMP-9 decreased after 8-week and this decrease was statistically significant (p<0.05).

Comparasion between the test and control group after therapy, a significant decrease in the GCF MMP-9 levels was found in the test group (p=0.01).

Discussion

As a result of our study; there was a significant decrease in clinical periodontal parameters and GCF volume in patients treated with gas ozone after SRP. A statistically significant reduction was observed IL-1β, IL-6, IL-10 and MMP-9 levels in groups, while MMP-9 levels were significantly decreased in the test group compared to the control group at 8-week.

In this study, it was observed that clinical periodontal parameters showed improvement after SRP according to baseline values after gas OT. In several studies, it has been determined that ozonized water treatment is more effective in improving clinical periodontal parameters compared to SRP alone in periodontitis therapy (9,10,12,13). In a study, Hayakumo et al. (9) reported that ozonized water as an adjunctive to SRP, improved the clinical periodontal parameters in patients with periodontitis after 4-week. Ranjith et al. (10) found that adjunctive ozone water irrigation resulted in significant advancement in all clinical parameters, outside of PD. Also a study performed by Kshitish et al. (12) showed that ozonized water as an adjunctive to SRP significantly improved the clinical periodontal parameters on the 18th day in patients with periodontitis. Moreover, Katti et al. (13) demonstrated that ozonized water as an adjunctive to SRP lead to a significant reduction in clinical periodontal parameters in 30-day evaluation. The results of our study is consistent with their studies.

There are also studies showing that OT has no effect on clinical periodontal parameters in addition to SRP (4,8,14-17). The results of these studies are not compatible with the results of our study. The differeces between our results and those of similar studies can be explained partially by the different types and concentration of ozone used, duration of application, presence or absence of control and type of control. Further randomized, double-blind and well-controlled clinical trials are required to obtain remarkable conclusions.

Ozonized water, ozonized oil and gas ozone are applied at different doses and times in *in vivo* and *in vitro* studies until now (5,8,16). In the present study, gas ozone was applied each tooth two times for consequtive three days for 60 sec, each tooth at 26 µg power (16,600 ppm at 60% power). Uraz et al. (15) reported that the split mouth study gas ozone application was 3 times during 30 s, 2100 ppm with 80%. Tasdemir et al. (16) in their study were applied gas ozone twice a week for 2 weeks, 75% power for 30s.

In the literature search, Uraz et al. (15) evaluated TNF-α, IL-1β, IL-6 in the GCF of patients with periodontitis for SRP + OT and SRP-alone. Following SRP-alone group TNF-α levels were significantly reduced at 4 week. IL-6 concentration

Table 1. Comparison of clinical parameters between groups, baseline and 8-week

	SRP + OT (n=25) (Mean + SD)	Between groups p* values	SRP alone (n=25) (Mean + SD)
PI baseline	2.26±0.38	NS	2.15±0.34
Within the group 8-week	p=0.000, 1.33±0.37	p<0.05	p=0.000, 1.62±0.37
GI baseline	2.50±0.44	NS	2.32±0.46
Within the group 8-week	p=0.000, 1.53±0.45	p<0.05	p=0.000, 1.86±0.42
PD (mm) baseline	3.88±0.48	NS	4.07±0.70
Within the group 8-week	p=0.000, 2.33±0.28	p<0.05	p=0.000, 2.60±0.43
CAL (mm) baseline	5.61±0.95	NS	6.23±1.26
Within the group 8-week	p=0.000, 4.12±0.39	p<0.05	p=0.000, 5.03±0.96
GCF volume (pl) baseline	0.65±0.20	NS	0.69+0.16
Within the group 8-week	p=0.000, 0.09±0.05	NS	p=0.000, 0.11±0.05

p*- Comparison of 8-weeks values of groups (t-student test). GCF: Gingival crevicular fluid, SRP: Scaling and root planing, OT: Ozone therapy, PI: Plaque index, GI: Gingival index, PD: Probing depth, BOP: Bleeding on probing, SD: Standard deviation, NS: Non-significant

Table 2. Comparison of biochemical parameters between groups, baseline and 8-week			
	SRP + OT (n=25) (mean + SD)	Between groups p* values	SRP alone (n=25) (mean + SD)
TNF-α (pg/mL)			
Baseline	1.63±0.32	NS	1.73±0.35
Within the group	NS		NS
8-week	1.87±0.51	NS	1.51±0.19
IL-1β (pg/mL)			
Baseline	131.52±53.49	NS	148.57±68.29
Within the group	p<0.05		p<0.001
8-week	1.53±0.45	p>0.05	59.64±40.35
IL-6 (pg/mL)			
Baseline	3.35±0.46	NS	3.51±1.18
Within the group	p<0.001		p<0.001
8-week	1.08±0.15	p>0.05	0.97±0.45
IL-10 (pg/mL)			
Baseline	1.40±0.58	NS	1.41±0.59
Within the group	p<0.05		p<0.05
8-week	0.71±0.21	p>0.05	0.82±0.35
MMP-9 (pg/mL)			
Baseline	93.73±48.90	NS	98.47±43.47
Within the group	p<0.05		p<0.05
8-week	14.37±5.91	p<0.05	45.30±14.61

p*- Comparison of 8-weeks values of groups (t-student test). SRP: Scaling and root planing, OT: Ozone therapy, TNF-α: Tumor necrosis factor-alpha, IL-1 β: Interleukin-1 beta, IL-6: Interleukin-6, MMP-9: Matrix metalloproteinases-9, SD: Standard deviation, NS: Non-significant

significantly decreased at 3-month follow-up in the SRP alone group. Tasdemir et al. (16) investigated reduction in IL-1 levels after both SRP + OT group and SRP alone group after 3-month, but the decrease was not statistically significant. No clinical studies were found topical application of ozone in the treatment of periodontitis to evaluate levels of IL-10 and MMP-9 in GCF, except for the study investigating the effects of ozonized water on salivary MMP-9 level. Skurska et al. (14) after treatment with ozonized water in patients with periodontitis, saliva was increased in MMP-9 level and saliva in MMP-9 level decreased in patients with aggressive periodontitis.

OT influences biomolecules that contain structures cysteine, methionine or histidine residues via ozone's oxidation power (18). Cysteine residue, in the homology region in domain-1 of MMPs, regulates the preservation of latency of the enzyme proform. The shift from the inactive into active form is subjected by degradation of cysteine thiol group that is caused by ozone-dependent reactions proteolytic dissociation or action of free radicals (18-20). It is considered that OT effects the structure of MMP-9 molecules, due to significant decrease in MMP-9 levels in the test group compared to the control group (p=0.01).

The present study has some limitations. First of all, ozone was determined to be toxic to human oral epithelial cells and gingival fibroblast. Secondly, each study was conducted under a different protocol as stated in the manufacturer's instructions for ozone application. Thus, the timing and power of ozone application were based on the manufacturer's suggestion, and not on scientific evidence. The last limitations of this study may be considered to have a small sample size and a short follow up period. Future studies with larger samples and longer follow-up periods to contrast various applications ozone agents for the treatment of periodontitis will give exciting results.

Conclusion

Medical ozone can be used as an adjunctive to SRP in the treatment of periodontal diseases. The most important issue in OT is the need for scientific studies to examine its long and short term effects by considering the parameters such as strength, duration and frequency of administration with all three ozone types.

Acknowledgements

This investigation was supported by Atatürk University Research Grant PN-2014/160. This study was presented as an oral presentation at the 46. Scientific Congress, Turkish Society of Periodontology, İzmir, Turkey. The authors report no conflicts of interest related to this study.

Ethics

Ethics Committee Approval: The study protocol was confirmed by the Ethics Committee of Atatürk University, Erzurum, Turkey (decision code: 24/2014, date: 13.10.2014).

Informed Consent: The signed consent form was obtained from all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.N.S.G., A.D., Concept: S.N.S.G., Design: A.D., Data Collection or Processing: S.N.S.G., N.Ö., M.A.G., Analysis or Interpretation: A.D., N.Ö., M.A.G., Literature Search: S.N.S.G., Writing: S.N.S.G., A.D.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This investigation was supported by Atatürk University Research Grant PN-2014/160.

References

1. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4: 1-6.
2. Aspriello SD, Piemontese M, Levrini L, Sauro S. Ultramorphology of the root surface subsequent to hand-ultrasonic simultaneous instrumentation during non-surgical periodontal treatments: an in vitro study. J Appl Oral Sci 2011; 19: 74-81.
3. Quirynen M, Teughels W, De Soete M, van Steenberghe D. Topical antiseptics and antibiotics in the initial therapy of chronic adult periodontitis: microbiological aspects. Periodontol 2000 2002; 28: 72-90.
4. Yılmaz S, Algan S, Gursoy H, Noyan U, Kuru BE, Kadir T. Evaluation of the clinical and antimicrobial effects of the Er:YAG laser or topical gaseous ozone as adjuncts to initial periodontal therapy. Photomed Laser Surg 2013; 31: 293-8.
5. Eick S, Tigan M, Sculean A. Effect of ozone on periodontopathogenic species--an in vitro study. Clin Oral Investig 2012; 16: 537-44.
6. Bocci VA. Scientific and medical aspects of ozone therapy. State of the art. Arch Med Res 2006; 37: 425-35.
7. Saglam E, Alinca SB, Celik TZ, Hacisalihoglu UP, Dogan MA. Evaluation of the effect of topical and systemic ozone application in periodontitis: an experimental study in rats. J Appl Oral Sci 2019; 28: e20190140.
8. Seydanur Dengizek E, Serkan D, Abubekir E, Aysun Bay K, Onder O, Arife C. Evaluating clinical and laboratory effects of ozone in non-surgical periodontal treatment: a randomized controlled trial. J Appl Oral Sci 2019; 27: e20180108.
9. Hayakumo S, Arakawa S, Mano Y, Izumi Y. Clinical and microbiological effects of ozone nano-bubble water irrigation as an adjunct to mechanical subgingival debridement in periodontitis patients in a randomized controlled trial. Clin Oral Investig 2013; 17: 379-88.
10. Ranjith A, Niranjana JM, Baiju KV. Adjunctive benefit of ozonized water irrigation with mechanical debridement in the management of Stage III periodontitis: A randomized controlled clinical and biochemical study. Int J Dent Hyg 2022; 20: 364-70.
11. Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornman KS, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. J Periodontol 2018; 89 Suppl 1: S1-8.
12. 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: 341-8.
13. Katti SS, Chava VK. Effect of Ozonised water on Chronic Periodontitis - A Clinical Study. J Int Oral Health 2013; 5: 79-84.
14. Skurska A, Pietruska MD, Paniczko-Drężek A, et al. Evaluation of the influence of ozonotherapy on the clinical parameters and MMP levels in patients with chronic and aggressive periodontitis. Adv Med Sci 2010; 55: 297-307.
15. Uraz A, Karaduman B, Isler SÇ, Gönen S, Çetiner D. Ozone application as adjunctive therapy in chronic periodontitis: Clinical, microbiological and biochemical aspects. J Dent Sci 2019; 14: 27-37.
16. Tasdemir Z, Oskaybas MN, Alkan AB, Cakmak O. The effects of ozone therapy on periodontal therapy: A randomized placebo-controlled clinical trial. Oral Dis 2019; 25: 1195-202.
17. 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: 37-43.
18. Bocci V. Is it true that ozone is always toxic? The end of a dogma. Toxicol Appl Pharmacol 2006; 216: 493-504.
19. Holmes J. Clinical reversal of root caries using ozone, double-blind, randomised, controlled 18-month trial. Gerodontology 2003; 20: 106-14.
20. Bocci V. Ozone as a bioregulator. Pharmacology and toxicology of ozonetherapy today. J Biol Regul Homeost Agents 1996; 10: 31-53.