

Application of Ozone Therapy in Dentistry



Dental Science

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ABSTRACT

In the field of dentistry, new treatment protocols are budding day by day to combat human ailments in a much better way. One such advancement is the application of ozone in dentistry. Ozone has several properties includes analgesic, immunostimulant and antimicrobial properties. This review throws light on the history, properties, biological actions and uses in the field of dentistry, contraindications of ozone.

Introduction

Ozone is a colorless gas form of oxygen. It is one of the most important gases in the stratosphere. Ozone is a naturally occurring gaseous molecule made up of three oxygen atoms [1]. It is a blue gas, with a strong odor and absorbs the harmful ultraviolet rays present in the light spectrum from the sun and protects the living creatures from the ultraviolet rays [2]. One molecule of ozone is equal to 3000-10,000 molecules of chlorine and kills pathogenic organisms 3500 times faster [3]. The word ozone comes from the greek 'ozein' meaning odorant. It surrounds the earth at an altitude of between 50,000 and 10,000 feet. Half life of ozone is 40 minutes at 20°C [4]. It has got a high-oxidation potential which is 1.5 times greater than chloride when used as an antimicrobial agent. It also stimulates blood circulation, and the immune response [5]. It is an unstable gas and quickly gives up nascent oxygen molecules to form oxygen gas. Due to the release of this nascent oxygen, it has been used in medicine for many years [6]. Studies have examined ozone's effect on dental caries and primary root caries, periodontitis, disinfection of denture surfaces, wound healing and as an antimicrobial, antiviral and antifungal agent in the oral cavity [7, 8].

History

The German chemist Christian Friedrich Schonbein (1840) of the University of Basel in Switzerland is regarded as the father of ozone therapy [9]. Ozone was first used in medicine in 1870 by Dr. C. Lender. Use of ozone was initially limited for industrial purpose mainly for disinfection of water. Dr. Charles Kenworthy, a Florida physician, in 1885, published his experiences with ozone in the Florida Medical Association Journal. In October 1893, Ousbaden, Holland became the first city to utilize a water treatment plant using ozone. The first reported medical application is the use of ozone for treating gaseous, post traumatic gangrene in German soldiers during First World War. In 1931, Dr. E. A. Fisch, a dentist used ozonated water for dental procedures and pioneered its use in medicine [10].

Biological actions

Ozone is a thermodynamically higher unstable compound that decomposes to pure oxygen depending on system conditions like temperature and pressure [11] [Figure-1].

a. Antimicrobial effect: The ozone oxidant potential induces the destruction of cellular walls and cytoplasmic membranes of bacteria [12]. Gram positive bacteria are

more sensitive to the action of ozone than gram negative. All viruses are susceptible to ozone; yet differ in their susceptibility [13]. Lipid enveloped viruses are especially sensitive to ozone [14]. An in vitro study in which evaluation of antibacterial effect of ozone was done after 2 months of disinfection with ozone on *Streptococcus mutans* and *Lactobacillus casei* was found to be more resistant to ozone [15].

b. Immunostimulating effect: Ozone influences cellular and humoral immune system. It stimulates proliferation of immunocompetent cells and synthesis of immunoglobulins. It also activates function of macrophages and increases sensitivity of microorganism to phagocytosis Ozone causes the synthesis of biologically active substances such as interleukins, leukotrienes and prostaglandins which is beneficial in reducing inflammation and wound healing [16].

c. Antihypoxic effect: Ozone brings about the rise of pO₂ in tissues and improves transportation of oxygen in blood, which results in change of cellular metabolism-activation of aerobic processes (Glycolysis, Krebs cycle, Beta-oxidation of fatty acids) and use of energetic resources. Ozone improves the metabolism of inflamed tissue by increasing their oxygenation and reducing total inflammatory processes.

d. Biosynthetic effect: It activates mechanisms of protein synthesis, increases amount of ribosome's and mitochondria in cells. These changes on the cellular level explain elevation of functional activity and regeneration potential of tissues and organs.

Ozone causes secretion of vasodilators such of NO, which is responsible of dilatation of arterioles and venules. It also activates angiogenesis by increasing the concentration of 2, 3 Diphosphoglycerate (2, 3-DPG). Ozone changes the configuration of erythrocytes, which enables them to return oxygen in the inflamed tissue.

Generators of Ozone

The first ozone generator was developed by Werner Von Siemens in Germany in 1857. There are several different techniques used to produce therapeutic grade ozone [2, 17, 18]. They are:

A. Ultraviolet system: Produces low concentration of ozone. It is used in esthetics, saunas and for air purification.

B. Corona discharge system: Produces high concentration of ozone. Most common system used in medical and dental field. It is easy to handle and it has high controlled ozone production.

C. Cold plasma system: Used in air and water purification.

Medical grade ozone is a mixture of pure oxygen and pure ozone in the ratio of 0.05% to 5% of ozone and 99.95% of oxygen. Due to the instability of the ozone molecule medical grade ozone must be prepared immediately before use. In order to control the decomposition of ozone into oxygen it can be associated with a vehicle with aqueous properties to promote the conversion more quickly or with a vehicle with more viscous properties to retard the conversion.

Routes of Ozone administration

1. Gaseous ozone: Topical administration of the gaseous form can be via an open system or via a sealing suction system as a prerequisite to avoid inhalation and adverse effects.

2. Ozonated water: Ozonated water has been shown to be effective against bacteria, fungi and viruses and is also less expensive compared to other chemical cleaners [19]. Gaseous ozone was shown to be more effective microbicide than the aqueous form and applied for three minutes, may be used as a dental disinfectant [20].

Because ozone gas has been found to have toxic effects if inhaled into the respiratory tract, ozonated water may be useful to control oral infection and various pathogens.

3. Ozonized oil: In addition to ozone application in its gaseous and aqueous form ozonized oil also seems extremely convenient.

Though gaseous ozone was shown to have more effective microbicidal properties than aqueous form, due to its toxic effects if inhaled, ozonated water is the most preferred form for use in dentistry.

Commercially available Ozone generators

CurOzone USA Inc. (Ontario, Canada) developed the HealOzone, which is now distributed by KaVo Dental (Kavo, Biberach, Germany), for use in dentistry [21] [Figure-1]. In clinical settings, ozone generators are used to produce ozone from medical oxygen via an electrical field that simulates the natural production of ozone at the time of lightning. This ozone is thereafter led to a hand piece fitted with a silicone cup. Differently shaped silicone cups are available that correspond to the form of various teeth and their surfaces [22].

Figure-1: Kavo healozone



Advantages

- Disinfectant
- Anti inflammatory
- Activation of intracellular metabolism of oral mucosa and dental wounds
- Improvement of regional circulation
- Stimulation of regenerative processes
- Hemostasis in capillary bleedings
- Painless procedure

Disadvantages

- Ozone toxicity if the level increases at 0.0007% per application
- Instability
- Not readily available

Indications

- Chronic or recurrent infections in the oral cavity [23]
- Prophylaxis and prevention of dental caries
- Remineralization of pit and fissure caries, root and smooth surface caries
- Bleaching of discolored root canal treated teeth
- Sterilization of cavities, root canals, periodontal pockets, herpetic lesion
- Desensitization of extremely sensitive tooth necks
- Pre-washing of surgical sites
- Plaque control
- Contamination control

Contraindications

The following are contraindications for use of ozone therapy [24].

- Pregnancy
- Glucose-6-phosphate-dehydrogenase deficiency
- Hyperthyroidism
- Severe anaemia
- Severe myasthenia
- Active hemorrhage
- Acute alcohol intoxication
- Recent myocardial infarction

Role of ozone in Dentistry

The use of ozone has been proposed in dentistry because of its antimicrobial, disinfectant, biocompatibility and healing properties.

Ozone therapy in Oral Surgery: Ozone has a positive influence on bone metabolism and reparative processes of the bone [25]. Use of ozonated water as a cooling and rinsing medium during surgical removal of third molar can reduce the occurrence of infection complications after the procedure [26]. In patients with chronic mandibular osteomyelitis it was observed that medical ozone exposure promoted more complete and rapid normalization of non specific resistance and T cellular immunity, thus accelerating clinical cure and reducing the incidence of complications [27]. Ozone therapy in the management of bone necrosis or in extraction sites during and after surgery in patients treated with bisphosphonates may stimulate cell proliferation and soft tissue healing [28]. When a combination therapy of a course of antibiotics, surgery and ozone therapy was given to patients with osteonecrosis of jaw in patients with multiple myeloma there was a decrease in both the incidence of osteoradionecrosis of the jaw and the extent of the lesions [29].

Ozone therapy in Conservative Dentistry: Ozone in gaseous and aqueous phase has a disruptive effect on cariogenic bacteria like streptococcus mutans and streptococ-

cus sobrinus. Ozone can convert pyruvic acid produced by cariogenic bacteria to acetic acid which can buffer the cariogenic acid and cause demineralization of the carious lesion. Effect of ozone is more in reverting noncavitated lesions when compared to the cavitated one [30]. Ozone treatment alone or combined with a remineralizing solution was found to be effective for remineralization of pit and fissure caries [31]. Assessment of influence of ozone gas and ozonated water application on resin-dentin bonds had shown that it can be used before bonding procedures with no deleterious effect on bond strength [32]. In one study on primary root caries lesion (PRCL) found that ozone application for either 10 or 20 sec dramatically reduced most of the microorganisms in PRCLs without any side effects recorded at recall intervals between 3 and 5.5 months [33].

Ozone therapy in Endodontics: The oxidative power of ozone characterizes it as an efficient antimicrobial. Ozone is one of the most powerful antimicrobial agents with enormous advantages to reduce the number of microorganisms in the root canal [34]. Its antimicrobial action has been demonstrated against bacterial strains such as *mycobacteria*, *streptococcus*, *pseudomonas aeruginosa*, *Escherichia coli*, *staphylococcus aureus*, *peptostreptococcus*, *enterococcus faecalis* and *candida albicans*. Notably, when the specimen was irrigated with sonication, ozonated water had the same antimicrobial activity as 2.5% NaOCl [19, 35].

Ozone therapy in Oral Medicine: Ozonated oil applied on herpes labialis and mandibular osteomyelitis demonstrated faster healing than conventional protocol [36]. In a study, patients with bilateral internal derangement of the temporomandibular joints, disc displacement with reduction in which direct injection of ozone gas in to the superior joint space was given. Each joint received 2 mL ozone-oxygen mixture. The injection was repeated 2 times per week for 3 weeks. Around 87% of the patients who received ozone gas injections in to the superior joint space either completely recovered or improved [37]. A case report by Shenberg and Blum [38] demonstrated gaseous and aqueous ozone therapy for treatment of mucositis secondary to chemotherapy and radiotherapy. The treatment protocol involved application of ozone in both aqueous and gaseous forms. Ozone in a gaseous form was provided at 40 to 60 sec per lesion. The gaseous ozone concentration was 2100 ppm, with a flow rate of ozone/air mix at 5 meter/sec. The aqueous solution of ozone bubbles and water is 2 to 4 ppm. The patient gargled with the aqueous solution for 1 to 2 minutes. Patient responded positively, enabling her to eat normally eliminating pain, medication and improved the quality of life. It is difficult to extrapolate for large patient cohorts from a single case study, so further research in to the therapeutic use of gaseous and aqueous ozone is indicated.

Ozone therapy in Prosthodontics: Microbial plaque accumulating on the dentures is composed of several microbial organisms, mainly *C. albicans*. Denture plaque control is essential for the prevention of denture stomatitis. The application of ozonated water may be useful in reducing the number of *C. albicans* on denture plate [39]. The use of ozone as denture cleaner is effective against Methicillin resistant *S. aureus* and viruses [40]. Ozone can be applied for cleaning the surface of removable partial denture alloy with little impact on the quality of alloy interms of reflectance, surface roughness and weight [41]. Direct exposure of gaseous ozone was a more effective microbicide compared with ozonated water. Therefore gaseous ozone can be clinically useful for disinfection of removable prosthesis [42]. There is also some evidence on the effectiveness of aqueous ozone application in adjunct to aminoalcohol for decontamination

of the implant surfaces [19].

Desensitization of sensitive root necks: Quick relief from root sensitivity has been reported following ozone spray for 60 seconds followed by mineral wash on to the exposed dentine in a repetitive manner. Smear layer present over the exposed root surface prevents the penetration of ionic calcium and fluorine deep in to the dentinal tubules. Ozone removes the smear layer, opens up the dentinal tubules broadens their diameter and then calcium and fluoride ions flow in to the tubules easily and effectively plug the dentinal tubules, thus preventing the fluid exchange through these tubules. Thus ozone can effectively terminate the root sensitivity problem within seconds [43].

Ozone in the treatment of periodontal diseases: The disinfection power of ozone makes the use of ozone in treatment of periodontitis. It is proved to be effective against gram positive and gram negative bacteria, viruses and fungi [44]. Ebensberger et al. [45] evaluated the effect of irrigation with ozonated water on the proliferation of cells in the periodontal ligament adhering to the root surfaces of 23 freshly extracted completely erupted third molars. The teeth were randomly treated by intensive irrigation with ozonated water for 2 minutes or irrigation with a sterile isotonic saline solution, serving as a control group. The periodontal cells of these teeth were studied immunohistochemically to mark proliferating cell nuclear antigen (PCNA). It was observed that the labeling index (the number of positive cells compared to the total number of cells suggesting enhancement of metabolism) was higher among the teeth irrigated with ozone (7.8% Vs 6.6%) however, the difference was not statistically significant ($p=0.24$). They concluded that the 2 minutes irrigation of the avulsed teeth with non isotonic ozonated water might lead not only to a mechanical cleansing, but also decontaminate the root surface, with no negative effect on periodontal cells remaining on the tooth surface. Another randomized controlled trial conducted in twenty-two subjects with periodontitis using ozone nano-bubble water irrigation as an adjunct to mechanical subgingival debridement revealed that this type of treatment may be a valuable adjunct to periodontal treatment [46].

Thanomsut et al. 2002 tested the effects of ozone treatment on cell growth and ultra-structural changes in bacteria (*Escherichia coli*, *Salmonella sp.*, *Staphylococcus aureus* and *Bacillus subtilis*). It was discovered that ozone at 0.167 mg/ml can be used to sterilize water, which is contaminated with up to 105 cfu/ml bacteria within 30 min. Destroying of bacterial cell membrane was observed, subsequently producing intercellular leakage and eventually causing cell lysis. Nevertheless, these ozone concentrations have no significant effect on the cell viability in bacterial cultures at higher concentrations of 106 and 107 cfu/ml [47]. Holmes in 2003, observed effect of kavohal ozone device on primary root carious lesion (PRCL) followed by professionally applied remineralizing solution containing Xylitol, fluoride, calcium, phosphate and zinc. This treatment modality was applied to 89 patients aged from 60 to 82 years. After 18 months 100% of ozone treated PRCLs had improved. In control group, where lesions were left without treatment, only 1 PRCL had improved. In 62% of cases the status remained leathery, while in 37% of PRCLs had worsened from leathery to soft [48]. Nagayoshi et al [19] tested the efficacy of three different concentration of ozone water (0.5, 2 and 4 mg/ml in distilled water) on the time dependent inactivation of cariogenic, periodontopathogenic and endodontopathogenic microbes in culture and biofilms. Gram negative bacteria such as porphyromonas endodontia-

lis and porphyromonas gingivalis were substantially more sensitive to ozonated water than gram positive oral streptococci and *C. albicans* in pure culture. Furthermore ozonated water had strong bactericidal activity against bacteria in plaque biofilm. In addition, ozonated water inhibited the accumulation of experimental dental plaque *in-vitro*.

Hems and Gulabivala, 2005 evaluated the potential of ozone as an antibacterial agent using *Enterococcus faecalis* as a test species. Ozone was used both gasiform (produced by purezone device), and aqueous (optimal concentrations 0.68 mg/l). It was concluded that ozone in solutions was antibacterial against planktonic *Enterococcus faecalis* after 240 seconds treatment. However it was not effective against *Enterococcus faecalis* cells in a biofilm unless they were displaced into the surrounding medium by agitation. Gaseous ozone was not effective on the *Enterococcus faecalis* biofilm [49]. Ramzy et al [50] irrigated the periodontal pockets by ozonated water in 22 patients suffering from aggressive periodontitis. Periodontal pockets were irrigated with 150 ml of ozonated water over 5 to 10 minutes once weekly, for a clinical four weeks study, using blunt tipped sterile plastic syringe. Highly significant improvement regarding pocket depth, plaque index, gingival index and bacterial count was recorded related to quadrants treated by scaling and root planning together with ozone application. They also reported significant reduction in bacterial count in sites treated with ozonated water. Huth et al [51] in their study declared that the aqueous form of ozone as a potential antiseptic agent, showed less toxicity than gaseous ozone or established antimicrobials (chlorhexidine di gluconate-CHX 2%, 0.2%; Sodium hypochlorite - NaOCl 5.25%, 2.25%; hydrogen peroxide 3%) under most conditions. Therefore, aqueous ozone fulfils optimal cell biological characteristics in terms of biocompatibility for oral application. Muller et al [52] compared the influence of ozone gas with photodynamic therapy and known antiseptic agents (2% chlorhexidine, 0.5% and 5% hypochlorite solutions) on a multispecies oral biofilm *in-vitro*. Gasiform ozone was produced by vacuum ozone delivery system kavoheal-ozone. They concluded that matrix-embedded microbial populations in biofilm are well protected towards antimicrobial agents. Only 5% hypochlorite solution was able to eliminate all bacteria effectively. Usage of gasiform ozone or PDT was not able to reduce bacteria in the biofilm. The Huth 2007 study establishes a condition in which aqueous ozone exerts inhibitory effects on the NF-kappa B system, suggesting that it has an anti inflammatory capacity. Kshithish and Laxman [53] conducted a randomized, double-blind, cross over split-mouth study on 16 patients suffering from generalized chronic periodontitis. The study period of 18 days was divided into two time intervals i.e. baseline 0 days to 7th day with a washout period of 4 days followed by a second time interval of 7 days. Subgingival irrigation of each half of the mouth with either ozone or chlorhexidine was done at different time intervals. They observed a higher percentage of reduction in plaque index (12%) gingival index (29%) and bleeding index (26%) using ozone irrigation as compared to chlorhexidine. The percentile reduction of *Aggregatibacter actinomycetemcomitans* (25%) using ozone was appreciable as compared to no change in *Aggregatibacter actinomycetemcomitans* occurrence using chlorhexidine. By using ozone and chlorhexidine, there was no antibacterial effect on *Porphyromonas Gingivalis* (Pg) and *Tannerella forsythensis*. The antifungal effect of ozone from baseline (37%) to 7th day (12.5%) was pronounced during the study period, unlike CHX, which did not demonstrate any antifungal effect. No antiviral property of ozone was observed. The antiviral efficacy of chlorhexidine was better than that of ozone. They concluded that despite the sub-

stantivity of chlorhexidine, the single irrigation of ozone is quite effective to inactivate microorganisms.

Huth et al in 2011 compared the effectiveness of ozone with that of the established antiseptic CHX, against periodontal microorganisms. There were no significant differences in the effectiveness of aqueous ozone compared with 2%CHX but they were more effective than 0.2% CHX. Therefore, high concentrated gaseous and aqueous ozone merit further investigation as antiseptics in periodontitis therapy [54]. Dodwad et al in 2011 compared the effect of oral irrigation with ozonated water, 0.2% Chlorhexidine and 10% Povidone iodine in patients with chronic periodontitis. The study concluded that local ozone application can serve as potent atraumatic, antimicrobial agent to treat periodontal disease non-surgically both for home care and professional practice. It may also serve as good tool during supportive periodontal therapy [55].

Osseointegration and Periimplantitis: The use of ozone around implants not only effectively sterilizes the surface of both implants and bone, but also initiates the reparative mechanisms allowing tissue regeneration around implant surface [56, 57]. Dental implant therapy has become the ultimate standard for replacement of missing teeth. An adequate and steady plaque control regimen must be ensured for the prevention of periimplantitis. Ozone kills the microorganisms causing periimplantitis and shows a positive wound healing effect due to the increase of tissue circulation. Gaseous ozone or ozonized water shows an increased healing compared to wound healing without ozone therapy. An *in vivo* study conducted by El Hadary et al [58] has evaluated that short-term administration of cyclosporine A, when administered with topical ozonated oil, may influence bone density and the quality of dental implant osseointegration. Therefore, topically applied ozonated oil may influence bone density and the quality of osseointegration around dental implants.

Conclusion:

Scientific research suggests ozone therapy has great potential in the treatment of various conditions encountered in dental practice looks promising. Treating patients with ozone therapy lessens the treatment time with an immense deal of variation and it eradicates the bacterial count more specifically. Advantage of ozone therapy is it is an atraumatic, biologically based treatment. There is good evidence of *in-vitro* biocompatibility of aqueous ozone with human oral epithelial cells, gingival fibroblast cells and periodontal cells. Further, the use of ozone is an easy and painless treatment modality. Further research in large scale is needed in this area, such research will surely pave way to the better future of dentistry.

REFERENCES

1. Das S. Application of ozone therapy in dentistry. IJDA. 2011;3:538-42.
2. Sujatha B, Kumar MG, Pratap GM, Vardhan R. Ozone therapy – A paradigm shift in dentistry. Health Sci. 2013;2:1-10.
3. Filippia A. The effect of ozonated water on epithelial wound healing. Douch Dent J. 2001;143:96-101.
4. Elvis AM, Ekta JS. Ozone therapy: A Clinical review. J Nat Sci Biol Med. 2011;2:66-70.
5. Meena A, Trivedi HP, Gupta M, Parvez S, Likhyan L. Therapeutic applications of ozonated products. Int J Dent Clin. 2011;3:68-9.
6. Bocca VA. Scientific and medical aspects of ozone therapy: State of the art. Arch Med Res. 2006;37:425-35.
7. Emerson M, Sprone OJ, Buck CE. Ozone inactivation of cell associated viruses. Appl Environ Microbiol. 1982;43:603-8.
8. Dyas A, Boughton BJ, Das BC. Ozone killing action against bacterial and fungal species. microbiological testing of a domestic ozone generator. J

- Clin pathol. 1983;36:1102-4.
9. Rubin MB. The history of ozone. The Schonbein period 1839-1868. Bull His Chem. 2001;26:40-56.
 10. Sameer M, Monica M. Ozone treating dental infections. Ind J Stomatol. 2011;2:256-9.
 11. Gupta G, Bansal M. Ozone therapy in periodontics. J Med Life. 2012;5:59-67.
 12. German IJ, Rodrigues AC, Andreo JC, Pomini KT, Ahmed FJ. Ozone therapy in dentistry-A systematic review. Int J Odontostomatology. 2013;7:267-78.
 13. Roy D, Wong PK, Engelbrecht RS, Chian ES. Mechanism of enteroviral inactivation by ozone. Appl Environ microbial. 1981;41:718-23.
 14. Burleson GR, Murray TM, Pollard M. Inactivation of viruses and bacteria by ozone with and without sonication. Appl microbial. 1975;29:340-4.
 15. Polydorou O, Halili A, Wittmer A, Pelz K, Hahn P. The antibacterial effect of gas ozone after 2 months of in vitro evaluation. Clin Oral Investi. 2012;16:545-50.
 16. Seidler V, Linetskiy K, Hubalkova H, Stankova H, Smucler R, Mazanek J. Ozone and its usage in general medicine and dentistry. Prague Med Rep. 2008;109:5-13.
 17. Makkar S, Makkar M. Ozone – treating dental infection. Indian J Stomatol. 2011;2:256-9.
 18. Komali G. Ozone therapy – A revolutionary non-invasive therapy in dentistry. Open Access Sci Rep. 2012;1:1-3.
 19. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. J Endod. 2004;30:778-81.
 20. Azarpazooch A, Limeback H. The application of ozone in dentistry: a systematic review of literature. J Dentist. 2008;36:104-16.
 21. Abhishek GM. Ozone: An emerging prospect in dentistry. Indian J Dent Sci. 2012;4:47-50.
 22. AbuNaba L, Shorman H, Holmes JK, Peterson L, Tagami J, Lynch E. The Revolution in Dentistry Evidence-based research into ozone treatment in dentistry: an overview. Quintessence Publishing. 2004;73-115.
 23. Baysan A, Lynch E. The use of ozone in dentistry and medicine. Prim dent care. 2005;12:47-52.
 24. Nogales CG, Ferrari PA, Kantorovich EO, Lage - Marquis JL. Ozone therapy in medicine and dentistry. J Contemp dent prac. 2008;4:75-84.
 25. Sanseverino ER. Knee joint disorders treated by oxygen ozone therapy. Eura Medico phys. 1989;25:163-70.
 26. Filipovic Z, Divic Z, Duski R, Grnjatovic N, Galic N, Prepeg D. Impact of ozone on healing after alveolectomy of impacted lower third molars. Saudi Med J. 2011;32:642-4.
 27. Agapov VS, Shulakov VV. Ozone therapy of chronic mandibular osteomyelitis. stomatologija (mosk) 2001;80:14-7.
 28. Shilpa RA, Reddy N, Dinapadu S, Reddy M, Pasari S. Role of ozone therapy in minimal intervention dentistry and endodontics . J Int Oral Health. 2013;5:102-8.
 29. Petrucci MT, Gallucci C. Role of ozone therapy in the treatment of osteonecrosis of the jaws in multiple myeloma patients. Haematologica 2009;1289-90.
 30. Huth KC, Paschos E, Brand K, Hickel R. Effect of ozone on non cavitated fissure carious lesion in permanent molars: A Controlled prospective clinical study. Am J Dent. 2005;18:223-8.
 31. Arabek D, Oztas N. Effectiveness of ozone with or without the additional use of remineralizing solution on non cavitated fissure carious lesion in permanent molars. Eur J Dent. 2011;5:393-9.
 32. Garcia EJ, Serrano AP, Urruchi WI, Deboni MC, Reis A, Grande RH, Loguericio AD. Influence of ozone gas and ozonated water application of dentin and bonded interfaces on resin-dentin bond strength. J Adhes Dent. 2012;14:363-70.
 33. Baysan A, Lynch E. Effect of ozone on the oral microbiota and clinical severity of primary root caries. Am J Dent. 2004;17:56-60.
 34. Cardoso MG, De Oliveira LD, Koga-Ito CY, Jorge AO. Effectiveness of ozonated water on candida albicans, enterococcus faecalis and endotoxins in rootcanals . Oral surg oral med oral path oral radiol Endo. 2008;105:85-91.
 35. Huth KC, Quirling M, Maier S, Kamereck K, Alkhayer M, Paschos E, et al. Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model. Int Endod J. 2009;42:3-13.
 36. Nogales CG, Ferrari PA, Kantorovich EO, Lage-Marques JL. Ozone therapy in medicine and dentistry. J Contemp Dent pract . 2008;4:75-4.
 37. Daif ET. Role of intraarticular ozone gas injection in the management of internal derangement of the temporomandibular joint. Oral Surg Oral Med Ral Path Oral Radiol Endod. 2012;113(6):10-4.
 38. ShenbergJ, Blum C. Gaseous and aqueous ozone therapy for treatment of mucositis secondary to chemotherapy/radiotherapy a case report. The Pain Practitioner. 2011;21:68-3.
 39. Arita M, Nagayoshi M. Microbicidal efficacy of ozonated water against candida albicans adhering to acrylic denture plates. Oral Microbial Immunol. 2005;20:206.
 40. Murakami H, Mizuguchi M. Effect of denture cleaner using ozone against methicillin resistant *staphylococcus aureus* and *E. coli* T1 phage. Dent mater. 2002;21:53-60.
 41. Suzuki T, Oizumi M. Influence of ozone on oxidation of dental alloys. Int J Prosthodont. 1999;12:179-83.
 42. Oizumi M, Suzuki T. In vitro testing of a denture cleaning method using ozone. J Med Dent sci. 1998;45:135-39.
 43. Garg R, Tandon S. Ozone: A new face of dentistry. The Internet Journal of Dental Science. 2009;7:2.
 44. Greene AK, Few BK, Serafini J. A comparison of ozonation and chlorination for the disinfection of stainless steel surfaces. J Dairy Sci. 1993;76(11):3617-20.
 45. Ebensberger U, Pohl Y, Fillipia A. PCNA- expression of cementoblasts and fibroblasts on the root surfaces after extra oral rinsing for decontamination Dental Traumatology. 2002;18:262.
 46. Hayakumo S, Arakawa S, Mano Y, Izumi Y. Clinical and microbiological effects of ozone nano-bubble water irrigation as an adjunct to mechanical sub gingival debridement in periodontitis patients in a randomized controlled trial. Clin Oral Investig .2013;17:379-88.
 47. Thanomsab B, Anupunpisit V, Chanphetch S, Watcharachaipong T, Poonkhum R, Srisukonth C. Effects of ozone treatment on cell growth and ultrastructural changes in bacteria. J Gen Appl Microbiol. 2002;48:193-9.
 48. Holmes J. Clinical reversal of root caries using ozone-double blind randomized controlled 18 month trial. Gerodontology. 2003;20:106-14.
 49. Hems RS, Gulabivala K, Ng YL, Ready D, Spratt DA. An *in-vitro* evaluation of the ability of ozone to kill a strain of *Enterococcus faecalis*. Int Endod J. 2005;38:22-9.
 50. Ramzy MI, Gomma HE, Mostafa MI, Zaki BM. Management of aggressive periodontitis using ozonated water. Egypt med J N RC. 2005;6(1):229-45.
 51. Huth KC, Jakob FM, Saugel B, Cappello C, Paschos E, Hollweck R, et al. Effect of ozone on oral cells compared with established antimicrobials. Eur J Oral Sci 2006;114:435-40.
 52. Muller P, Guggenheim B, Schmidlin PR. Efficacy of gasiform ozone and photodynamic therapy on a multispecies oral biofilm in vitro. Eur J Oral Sci. 2007;115:77-80.
 53. 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;21:341-48
 54. Huth KC, Quirling M, Lenzke S, Paschos E, Kemereck K, Brand K, et al. Effectiveness of ozone against periodontal pathogenic microorganisms. Eur J Oral Sci. 2011;119:204-10.
 55. Dodwad V, Gupta S, Kumar K, Sethi M, Masamatti S. Changing paradigm in pocket therapy-ozone versus conventional irrigation. Int J Public Health Dent. 2011;2:7-12.
 56. Kazancioglu, Kurklu E, Ezirganli S. Effects of ozone therapy on pain, swelling, and trismus following third molar surgery. Int J Oral Maxillofac Surg 2013;42:537-43.
 57. Mohammadi Z, Shalavi S, Soltani MK, Asgary S. A review of the properties and applications of ozone in endodontics- An update. Iran Endod J. 2013;8:40-3.
 58. El Hadary AA, Yassin HH, Mekhemer ST, Holmes JC, Grootveld M. Evaluation of the effect of ozonated plant oils on the quality of osseointegration of dental implants under the influence of cyclosporine A: an in vivo study. J Oral Implantol. 2011;37:247-57.