Efficacy of ozone therapy on *Porphyromonas gingivalis* count in chronic periodontitis: An in vivo study

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Abstract

**Background:** Periodontitis is a chronic inflammation that destroys tissues and attachment apparatus, leading to loss of teeth. *Porphyromonas gingivalis* is essentially absent in healthy periodontal tissues while it is isolated at a significant amount in periodontitis. Thus, *P. gingivalis* is considered as a major microbial etiologic factor for periodontitis. Several therapeutic regimens including a combination of surgical and non-surgical techniques are available to control the spread of pathogenic microbes in the periodontal tissues. Most of these treatment options although effective have fallen short on establishing as the therapeutic gold standards. Thus, it is vital to explore novel therapeutic modalities for treating periodontitis effectively. Given the recent success of the use of ozone therapy as an antimicrobial, the present study explores its potential role in effectively controlling the *P. gingivalis* count in cases of chronic periodontitis.

**Materials and Methods:** A total of 30 adult patients diagnosed clinically as chronic periodontitis with gingival inflammation and pocket depth ≥5 mm were included in the study. Patients with systemic conditions and/or other oral lesions/diseases were excluded from the study. The gingival crevicular fluid of patients was collected using paper points. The collected samples were assessed for colony-forming units of *P. gingivalis*. All patients positive for the microbe were subjected to oral scaling followed by the first set of ozone therapy following which they were immediately evaluated for a microbial count. Cases which were positive post first set of ozone therapy were subjected to a second set of ozone therapy following which they were immediately assessed for the microbial count.

**Results:** Of the 30 chronic periodontitis cases, only 12 (40%) showed the presence of *P. gingivalis*. These 12 patients were treated to a round of ozone therapy. The follow-up showed that of the 12 patients, six patients were negative for *P. gingivalis* and six patients were positive but had significantly reduced the count. Following a second round of ozone therapy for the six positive cases, five cases were negative and one case was positive but with a significantly reduced count.

**Conclusion:** The results show that a single round of ozone therapy led to a substantial reduction in all the chronic periodontitis cases positive for *P. gingivalis*. The second round led to effectively inhibiting all but one case of the study sample. The sample which remained positive despite two rounds of ozone therapy had a relatively higher microbial count, to begin with. Thus, to conclude in the limited sample size of the present study, ozone therapy has shown to effectively control *P. gingivalis* count and could indeed be used as an adjunct to conventional treatment modalities for chronic periodontitis. Further, multicenter prospective studies are needed to confirm the effectiveness of ozone therapy on a larger scale.
Introduction

Periodontitis represents chronic inflammation, leading to the destruction of the periodontium culminating with the loss of teeth. Most cases of periodontitis are accompanied by the presence of anaerobic and microaerophilic bacteria accumulating in the subgingival tissues.\(^1\)\(^2\) Although the mild form of periodontitis is relatively common, especially in older age groups, about 10–15% of the general population are afflicted with severe periodontitis resulting in debilitating oral manifestations. The onset of periodontitis is marked by a dramatic shift in the microbial flora of the periodontium. *Streptococcus* and *Actinomyces* which predominate in the healthy periodontium are replaced by *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* in periodontitis.\(^3\) The enzymes produced by these microorganisms lyse the host proteins resulting in tissue destruction. Eradication of this pathological microbiota has shown to halt the progress of the periodontal destruction.\(^4\)\(^5\) Although several treatment modalities including a combination of surgical and medicinal interventions have been successfully used to treat periodontitis, most cases have shown to recur. Most often the recurrence is marked by the more rapid progression of the disease and refractoriness to early treatment modalities. Thus, it is vital to prevent recurrence of periodontitis ensuring total eradication of the pathogenic bacteria. Surgical interventions although ensure the removal of diseased tissues often lead to severe compromise on the periodontium. Thus, a therapy capable of successfully halting disease progress without compromising the periodontium is the need of the hour. Recent studies have shown the use of ozonized solutions for treating infections including periodontitis.\(^6\) The antimicrobial effect of ozone not only restricts the spread of infection but also aids in the healing of the damaged tissue. At present, there is a lack of data as to the effect of ozone therapy on microbial colonies in periodontitis. Thus, the present study aims to evaluate the effect of ozone therapy on *P. gingivalis* in the gingival crevicular fluid (GCF) of chronic periodontitis patients.

Materials and Methods

Source of data

Clearance was obtained from the institutional ethics committee and consent was obtained from the patients included in the study. Patients diagnosed with chronic periodontitis were selected from the outpatient Department of Periodontology and Oral Implantology of Dr. D. Y. Patil College and Hospital, Pimpri, Pune. Patients periodontal status was confirmed using CPITN index and patients with 3 or 4 scores were selected for the study.

Inclusion criteria

Male and female adults with chronic periodontitis with pocket depth of ≥5 mm were included in the study.

Exclusion criteria

The following criteria were excluded from the study:
- Patients who have received antimicrobial and anti-inflammatory therapy in previous 6 months
- Patients giving a history of aggressive periodontitis
- Patients with other oral/systemic conditions and infections
- Patients who are smokers and tobacco consumers
- Patients with a history of periodontal treatment within the last 6 months
- Pregnant patients

Sample size

The sample consisted of 30 patients diagnosed as chronic periodontitis with a pocket depth of ≥5 mm.

Primary sample collection

Supragingival plaque was removed to prevent cross-contamination, following which paper points “ISO 45” were placed in the three deepest periodontal pockets of each patient to collect the GCF. The paper points were placed kept in situ for 30 s each. The collected GCF samples were immersed in reduced transport fluid (RTF) and transported to the laboratory within 24 h. It was then processed and colony-forming units (CFUs) for *P. gingivalis* were determined.

Patients were subjected to oral scaling followed by the first set of ozone therapy. Each set of ozone therapy consisted of irrigating the periodontal pockets with a blunt tipped sterile plastic syringe with 150 ml of ozonized water for over 5–10 min once weekly for 3 weeks. Following each set of ozone therapy, the pre-ozone and the post-ozone GCF samples were sent to a laboratory for further microbiological evaluation within 24 h. Microbiological analysis was done in the Department of Microbiology and Immunology, Maratha Mandal G. Halgekar Institute of Dental Sciences and Research Centre, Belgium.

Laboratory procedures

GCF samples received in the RTF transport medium were first vortexed. Then, the samples were inoculated in the culture medium in the enriched and selective medium. For *P. gingivalis*, GCF samples were inoculated in blood agar which was used as an enriched media along with Brucella agar Hemin and Vitamin K. Kanamycin blood agar was used as the anaerobic selective medium. As *P. gingivalis* is strictly anaerobe, the sample was immediately incubated at 37°C for 3–4 days in an anaerobic jar.

Then, after completion of incubation, the plants are removed and noted for colony characters. As a consequence of storing iron in the form of protopheme on the surface of *P. gingivalis*, the bacteria can be distinguished from Bacteroidaceae family by the formation of black-pigmented colonies on blood agar plates.\(^7\) Colonies appear as wet, mucoid showing shiny surface with smooth edges. The required organism is identified and colony count is done for qualification. These organisms are confirmed by Gram staining and key biochemicals such as glucose, sucrose, cellobiose, and arabinose.
Results

The present study was conducted to assess the efficacy of ozone therapy on *P. gingivalis* count in a GCF in patients with chronic periodontitis. In the study, 30 chronic periodontitis patients were selected which comprised 16 males and 14 females. The number of patients with CPTIN index 3 and 4 was 7 and 23, respectively. The sequence of GCF collections and analysis is summarized in Figure 1.

*P. gingivalis* was isolated in 12 of 30, that is, 40% of patients with periodontitis. The positive patients were subjected to oral scaling and the first set of ozone therapy followed by the immediate collection of GCF. The pre- and post-ozone therapy GCF samples were sent for microbial assessment. The average baseline *P. gingivalis* levels before ozone therapy were in the range of \(4.68 \times 10^5 \pm 3.93 \times 10^5\) CFU, whereas after ozone therapy, the values were significantly reduced to \(2.04 \times 10^5 \pm 0.19 \times 10^5\) CFU. The results showed that the *P. gingivalis* CFU count was completely negative for six patients, and in the rest, the microbe was positive but showed a significantly reduced count. After 6–7 weeks following the first set of ozone therapy, the subjects positive for *P. gingivalis* were subjected to a second set of ozone therapy followed immediately by GCF collection and assessment. The results showed that five cases were negative for the microbe while only one case was positive but showed a significantly reduced count than previous reading. The one case which was positive despite two sets of ozone therapy was found to have a relatively higher *P. gingivalis* from the start. The mean number of patients with *P. gingivalis* before ozone therapy was 32 ± 45.99. The mean number of patients after the first set of ozone therapy was 16.25 ± 34.01 and mean number of patients after the second set of ozone therapy was 4.1 ± 14.43. Mann–Whitney U-test was used for statistical analysis, *P* value of which was found out to be <0.05, indicating a significant decrease in *P. gingivalis* levels following each set of ozone therapy. Table 1 summarizes the results.

Discussion

The prime etiological agents in periodontal disease are bacteria. The most common oral pathologies such as dental caries, periodontal disease, and peri-implantation have been correlated with the formation and development of an oral biofilm, and the specific microorganisms present inside it.\[8,9\]

Conventional methods for periodontal therapy include the mechanical removal of biofilm and adjunctive use of various antibiotics.\[10,11\] Along with mechanical removal, the need of the hour is to find various means of reducing bacterial load. Given the increasing resistance to common antibacterial agents, it is vital to find an alternative therapeutic modality capable of effectively controlling the bacteria count. Due to its antimicrobial, disinfectant, biocompatibility, and healing properties, there is a surge in the increased use of ozone in various fields of medicine including oral health care. It is being effectively used in areas such as early carious lesions, cavities sterilization, periodontal pockets, wound healing in ulceraions and herpetic lesions, bleaching of discolored root canal treated teeth, and desensitizing sensitive teeth.\[12\] It is also used as a rinsing solution for avulsed teeth and as a denture cleaner.\[13,14\] Its use as a possible alternative antiseptic agent is also currently being discussed in dentistry.

Ozone acts on cells by ozonolysis of dual bonds of the cytoplasmic membrane. Its secondary oxidants effects lead to the modification of intracellular contents. The action of ozone is selective to microbial cells; it does not damage other cells of the human body due to its antioxidative ability. It is also effective against antibiotics resistant strains and acidic pH increases its antimicrobial activity.\[15\] It combines with bacterial cell membranes containing cysteine, cysteine, methionine, and

![Figure 1: Sequence of gingival crevicular fluid collections and assessment](image-url)
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The use of mouth rinses. The use of ozone therapy to reduce the microbial load in the oral cavity such as oral prophylaxis and periodontal treatments. Periodontal diseases contain more than 500 distinct microbial species. The growing microbial resistance has led to the use of higher concentrations of antibiotics which, in turn, leads to side effects including drastic alterations to the host beneficial microflora. Thus, a therapeutic modality

Table 1: Summary of the results

| Treatment phases | Number of patients with P. gingivalis | Mean | Standard deviation | Mann–Whitney U-test |
|------------------|--------------------------------------|------|--------------------|---------------------|
| Before treatment 1 | 12                                   | 32   | 45.99              | P < 0.05            |
| After treatment 1 | 6                                    | 16.25| 34.01              |                     |
| After treatment 2 | 1                                    | 4.16 | 14.43              |                     |

histidine and attacks the thiol groups of cysteine. Gram-positive bacteria are more sensitive to ozone therapy than Gram-negative bacteria.\[16\] It stimulates the proliferation of immunocompetent, thereby increasing the sensitivity of microorganisms to phagocytosis by activating the function of macrophages. Special messengers called cytokines are produced by immune cells as a response to this activation which helps in activating other immune cells to resist diseases.\[15\]

In the present study, of 30 chronic periodontitis patients, P. gingivalis was isolated in 40% of cases. After the first set of ozone therapy, 50% of patients were negative for P. gingivalis, whereas the rest showed positivity but with a significantly reduced microbial load. The second ozone therapy was given after 6–7 weeks after which 83.33% of patients were negative for P. gingivalis colonies while one patient was positive but showed a significant decrease in P. gingivalis count.

In an in vitro study conducted by Katti et al., anaerobic bacterial colonies, grown on culture plates, were tested against saline and ozone treated water for a reduction in the number of colonies. They observed that the ozone therapy was highly effective in reducing Gram-negative organisms like P. gingivalis and also that the sensitivity of P. gingivalis is more to ozonized water compared to Fusobacterium nucleatum in pure cultures. According to them, this could be due to the rupture of the cell wall membrane of the organism, leading to rapid inactivation of microorganisms.\[17\]

Hauser-Gerspach et al. stated that gaseous ozone showed selective efficacy to reduce adherent bacteria on titanium and zirconium without affecting adherence and proliferation of osteoblastic cells. P. gingivalis was eliminated by ozone from all surfaces within 24 s to levels below the detection limit (99.94%).\[18\]

Nagayoshi et al., 2004, studied the efficacy of ozonized water on survival and permeability of microorganisms and found that the water had strong bactericidal activity against bacteria in plaque biofilm. In vitro results of the study showed a reduction in dental plaque. Gram-negative bacteria such as Porphyromonas endodontalis and P. gingivalis were more sensitive to ozonized water than Gram-positive oral Streptococci and Candida albicans.\[19\]

Bezirtzoglou et al. found that the microbial load decreased gradually as an effect of ozone treatment. However, a bacterial regrowth was effective following short ozone period. Decontamination was complete after an extended exposure to ozone for 30 min.\[20\]

There are various treatment modalities to reduce the microbial load in the oral cavity such as oral prophylaxis and use of mouth rinses. The use of ozone therapy to reduce the microbial count is the new emerging technique in dentistry. Ozone gas insufflation is the most effective way to reduce microbial activity/count. In this study, chronic periodontitis patients were treated with ozone therapy to evaluate its effect on P. gingivalis count. There was a significant decrease in the count following each set of ozone therapy. After the second set of ozone therapy, there all but one patient was positive for the microbe. The positive patient had a relatively higher count from the start of the study.

Very few studies have evaluated the effect of ozone therapy on the microbial count of P. gingivalis in periodontitis patients, with most of these studies being in vitro. The present study is an in vivo analysis of the effect of ozone therapy in controlling the P. gingivalis count in patients with periodontitis. The results are similar to the studies done in vitro, that is, there was a significant decrease in CFU count following ozone therapy. This indicates that ozone is effective in killing the microorganisms by degradation of their cell membrane both in vitro and in vivo.

According to the literature, 70–80% of patients with chronic periodontitis have P. gingivalis as the main causative agent. However, in our study, only 12 of 30, that is, 40% of chronic periodontitis patients showed the presence of P. gingivalis CFU. Remaining 60% of patients did not show the presence of P. gingivalis. The major reasons for discrepancies as to the prevalence of P. gingivalis in periodontitis could be due to low sensitivities of culturing techniques and sampling of a limited number of oral sites causing an underestimate of the organism’s true prevalence.\[15\] As ozone therapy is painless, the acceptability and patient compliance for the treatment modality was better in the present study. As plaque formation is a continuous process in the oral cavity, the time required for accumulation of organisms (P. gingivalis) needs to be known. As to the best of our knowledge, very few studies have evaluated the same. It is difficult to exactly recall patient so as to prevent further plaque accumulation and disease progression. In our study, the follow-up period was limited to only a few weeks. Thus, a further study evaluating the P. gingivalis CFU for a longer follow-up period could provide valuable insights into the pattern of P. gingivalis regrowth following therapeutic interventions.

Conclusion

Ozone therapy has excellent medicinal value in the different periodontal treatments. Periodontal diseases contain more than
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How to cite this article: Londhe S, Bagul N, Gupta AA, Bhagat P, Kheur S. Efficacy of ozone therapy on Porphyromonas gingivalis count in chronic periodontitis: An in vivo study. J Oral Dis Markers 2018;2:30-34.