The Effect of Exposure Times of Gaseous Ozone on Bacterial Growth of Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis isolated from Sub Gingival Plaque Patients with Chronic Periodontitis

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ABSTRACT

Introduction: Periodontal disease in all forms occurs due to mix of microbial infections with specific types of bacterial pathogen. Strong evidence has implicated Porphyromonas gingivalis (P.g), and Aggregatibacter actinomycetemcomitans (A.a) to the pathogenesis of chronic periodontitis. Ozone in dentistry is currently being a possible alternative antiseptic agent due to its highly antimicrobial effectiveness in the treatment of oral diseases. The aim of this study was to determine whether different exposure times of gaseous ozone (1-10 minutes) with a dose of 218 ppm/W-air. Methods: Sub gingival plaque samples were collected from ten patients with chronic periodontitis with pocket depth (PDP) > 6 mm. The subgingival plaque was spread on selective agar media for both (A. a) and (P.g) and incubated anaerobically using an anaerobic jar and anaerobic gas packs in the incubator for 72 hours at 37°C. Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis colonies were diagnosed according to their morphological characteristics. The effect of exposure time (1-10 minutes) of gaseous ozone (218 ppm / W.air) on (A.a) and (P.g) growth on agar surface was carried out. Results: eliminates the growth a bacterial culture of (A. a) and (P.g) in Petri dishes. The inactivation effect of ozone was observed on both (A. a), and (P.g) colonies. After ozone exposure, the numbers of bacterial colonies on the surface agar decreased in a time dependent manner and the colony growth was no longer detected at 7, and 4 minutes of treatment against (A. a) and (P.g) respectively. Porphyromonas gingivalis (bacteria were much more sensitive toward ozone gas compared to (A.a). Conclusion: the results of this study showed that in vitro, exposure to gaseous ozone (218 ppm/W-air) for 4 and 7 minutes, effectively inhibited the bacterial growth of (P.g) and (A.a) respectively.

KEYWORDS: Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Periodontitis, Sub Gingival Plaque

INTRODUCTION

Periodontal diseases are bacterial infections of gingiva and supporting structures of teeth (periodontal ligament and bone) which hold the teeth in the alveolar bone.¹ Bacterial plaque are the main causes of periodontal disease which is colorless and sticky layer found on teeth, and more than 500 different bacterial types are able to colonize the adult oral cavity.² Chronic periodontitis is the most prevalent form of periodontitis, and it generally demonstrates the characteristics of a slowly progressing inflammatory disease. However, systemic, and environmental factors (diabetes mellitus, smoking) may modify the host’s immune response to the dental biofilm so that periodontal destruction becomes more progressive. Although chronic periodontitis is most frequently observed in adults, but it can also occur in children and adolescents due to chronic plaque and calculus accumulation.³ Generally, periodontal disease in all forms occurs due to mix of microbial infections with specific types of bacterial pathogen.⁴,⁵ and many different bacterial types are able to colonize the adult mouth.⁶ Over 700 different phenotypes of the oral bacterial microbiome includes approximately 400 species found in subgingival plaque.⁷,⁸ The gram negative anaerobic rods and spirochetes are more to be found in the subgingival plaque.⁹ Strong evidence has implicated Porphyromonas gingivalis (P.g)¹⁰,¹¹, and Aggregatibacter actinomycetemcomitans (A.a)¹²,¹³,¹⁴ to the pathogenesis of chronic periodontitis. Recently, ozone therapy has been used in various treatment modalities like in the field of medicine, dentistry, veterinary, food industry, water treatment, etc. Ozone in dentistry is currently being a possible alternative antiseptic agent due to its highly antimicrobial effect without causing any drug resistance and that has been noted in water purification and food preservation.¹⁴,¹⁵ Recent investigations have been

reported for both gaseous and aqueous ozone for their antimicrobial effects on oral pathogens, and the effectiveness of ozone in the treatment of oral diseases is currently a subject of intense research.\(^{16,17}\) The aim of this study was to determine whether different exposure times of gaseous ozone with a dose of 218 ppm/W.Air, applied to Petri dishes containing a bacterial culture of (A. a) or (P.g) eliminates their growth.

**MATERIALS AND METHODS**

The subgingival plaque samples were collected from ten patients with chronic periodontitis attending the clinic at the Department of periodontics at the teaching hospital of College of Dentistry / Baghdad University. Subgingival Plaque samples were collected from patients with periodontal pocket depth (PPD) > 6 mm. With age range was (35-55) years old. The patients with chronic periodontitis have least four to six mm depth with attachment loss of one to two mm. The tooth was cleaned and the supragingival plaque removed; the area was isolated with cotton rolls to prevent contamination from saliva. Periodontal gracy curettes were used to collect subgingival plaque from the deepest point of the pocket, the procedure was done very carefully to avoid touching adjacent tissue. The subgingival plaque was put on a swap that was inserted immediately in transfer media to preserve the sample which is then spread on selective agar media for both A. a and P.g and incubated anaerobically using an anaerobic jar and anaerobic gas packs in the incubator for 72 hours at 37°C. All subgingival plaque samples were tested positive for the presence of A.a and Pg. by bacteriological and biochemical test. *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* colonies were examined directly on the selective media plates and under a dissecting microscope (magnification x15) and diagnosed according to their morphological characteristics on the agar plates, and according to the description cited by.\(^{9}\) The ozone generator OLYMPIC. III (600mg/hr.) was used to generate gaseous ozone (214 ppm/W.air) and with special aeration stone for generation ozonated water (0.6ppm). The Ozone Generator was operated according to the manufacturer's instructions (Fig.1). This Ozone generator has four-time grades (3, 5, 10, and 15 minutes), realizing various functions of disinfection and sterilization by setting different times. In this study, a small plastic jar was used. The plastic cover has one ozone gas inlet port to inject the ozone gas and distribute it evenly throughout the jar, and one gas outlet for the release of the ozone gas. The ozone generator was fed with 1 LPM of dry compressed air as a feed gas. Ionized air was bypassed around the agar plates to supply a total airflow of ozone of 218 ppm/W.air. The ozone gas/dry air mixture flowed into the jar for different times (minutes) according to the experimental design. The ozone level in the plastic jar was kept consistent during the time periods by adjusting the outlet port (Fig 2). The effect of exposure time (1-10 minutes) of gaseous ozone (218 ppm / W.air) on (A.a) and (P.g) growth on agar surface was carried out by adding 5.0ml (10^6 CFU/ml) of bacterial broth to a test tube. Using a sterilized Loop, a loopful of bacterial growth was streaked on agar petri plate (A.A. /P.g. agar). The plate was covered by its lid and placed in the jar, and the jar was covered by its plastic cover. Each plate was exposed to the ozone for a specific time, and then incubated anaerobically at 37 °C for 24.48 hrs. The bacterial growth corresponding to each exposure time on each plate was performed visually and recorded.\(^{18}\) Plates showing no bacterial growth mean highly efficient exposure time of the gas ozone.

**RESULTS**

The results obtained for the qualitative evaluation of gaseous ozone (218 ppm/W.Air), of different exposure times (1-10 minutes) on the bacterial growth is presented in Figs 3, 4 and Table 1. The inactivation effect of ozone was observed on both (A. a), and (P.g) colonies. After ozone exposure, the numbers of bacterial colonies on the surface agar decreased in a time dependent manner and the colonies growth was no longer detected in 7, and 4 minutes of treatment against (A. a) and (P.g) respectively. The analysis of the results (Table 1) verified that (P.g) bacteria were much more sensitive toward ozone gas compared to (A.a).
investigated the use of gaseous ozone on bacteria adhering to implant surfaces and showed a selective reduction in bacteria, and concluding that gaseous ozone may have a role in treatment of periimplantitis. Pereira et al., reported that application of a gaseous O3/O2 mixture (0.4%/99.6%) for 1 h, at constant pressure and flow (11 mm Hg and 2 L/min, respectively) and controlled temperature, in plates containing 10^7 CFU/mL of E. coli, S. aureus, and P. aeruginosa led to total inhibition of growth of these bacteria. Fontes et al., concluded that the application of a low dose of gaseous ozone (dose of 20 µg of O3/ml in a gaseous O3/O2 mixture) for 5 minutes completely prevented in vitro the growth of gram positive and negative pathogenic bacteria commonly present in patients with severe nosocomial infections, with known resistance to antibiotics. In study done by Selçuk et. al., they compared between two groups one with scaling and Er:YAG laser the other with scaling and topical gaseous ozone and the results shows that ozone has an antimicrobial effect equivalent to that of the Er:YAG laser.

## DISCUSSION

Ozone is a potent oxidant and an important disinfectant, acting on microorganisms by means of oxidation of their biological material. It has been reported that O3 can be employed as a bactericidal agent under various forms, such as ozonized saline solution, ozonized water, ozonized oil, and more frequently the gaseous O3/O2 mixture. Gaseous ozone has also been potentially considered for the disinfection of the hospital environment, which can be a source of microorganisms for patients. The results of this study showed that gaseous ozone (218 ppm/W-air) was highly effective in eliminating of both (A.a) and (P.g). (7 and 4 minutes respectively). The sensitivity of (P.g) towards gaseous ozone, as demonstrated by lower killing time (4 minutes), was higher than that observed for (A.a) (killing time 7 minutes). These results could be explained by the conclusions reached by who investigated the use of gaseous ozone on bacteria adhering to implant surfaces and showed a selective reduction in bacteria, and concluding that gaseous ozone may have a role in treatment of periimplantitis. Pereira et al., reported that application of a gaseous O3/O2 mixture (0.4%/99.6%) for 1 h, at constant pressure and flow (11 mm Hg and 2 L/min, respectively) and controlled temperature, in plates containing 10^7 CFU/mL of E. coli, S. aureus, and P. aeruginosa led to total inhibition of growth of these bacteria. Fontes et al., concluded that the application of a low dose of gaseous ozone (dose of 20 µg of O3/ml in a gaseous O3/O2 mixture) for 5 minutes completely prevented in vitro the growth of gram positive and negative pathogenic bacteria commonly present in patients with severe nosocomial infections, with known resistance to antibiotics. In study done by Selçuk et. al., they compared between two groups one with scaling and Er:YAG laser the other with scaling and topical gaseous ozone and the results shows that ozone has an antimicrobial effect equivalent to that of the Er:YAG laser.

## CONCLUSION

The results showed that in vitro, exposure to gaseous ozone (218 ppm/W.Air) for 4 and 7 minutes, effectively inhibited the bacterial growth of Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis isolated from subgingival plaque in patients with chronic periodontitis respectively.

## REFERENCES

Ahmed BZ et al.: Effect of Gaseous Ozone on Bacterial Growth in Patients with Chronic Periodontitis


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