Republic of Iraq Ministry of Higher Education & Scientific Research University of Baghdad College of dentistry



## Antibacterial Effect of ozonated water, ozonated gas and ozonated olive oil Against Anaerobic Periodontal Pathogens (An *in vitro* study)

A Thesis

Submitted to the council of college of dentistry / university of Baghdad in partial fulfillment of the requirement for the award of the degree of Master of Science in Periodontics

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Baghdad-Iraq

2016 A.D.

1437 A.H.

## Abstract

**Background:** chronic Periodontitis is considered to be an inflammatory diseases and is characterized by the damaging the connective tissue and supporting bone due to secondary infection by periodontal bacteria. Substantial evidence has implicated *Porphyromonas gingivalis* (P.g) and *Aggregatibacter actinomycetemcomitans* (A. a) to be the pathogenesis of the chronic periodontitis.

Ozone is a powerful antimicrobial agent which possess an effect against bacteria, fungi, protozoa, and viruses by damaging its cytoplasmic membrane.

**Aims of study:** The Aims of this study were to evaluate and compare the antimicrobial efficiency of ozonated water, Ozonated gas, ozonated olive oil, and 0.2% chlorhexidine against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* isolated from patients with chronic Periodontitis. And study the antibacterial effect of different exposure time interval of ozonated water, Ozonated gas on the isolated bacteria.

**Material and mathods:** Subgingival Plaque samples were collected from 10 systemically healthy patients with chronic periodontitis. With range age was (35-55) years old; the subgingival plaque samples were collected from the periodontal pocket with probing pocket depth (PPD) of >6 mm depth with loss of attachment about one to two mm (CAL). All subgingival plaque samples were tested positive for the presence of A.a and Pg. by bacteriological and biochemical test.

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). Ozonated Olive Oil gel (1 ppm) concentration was used

**Results**: The antimicrobial activity (Minimal Inhibitory Concentration MIC and Minimal Bactericidal Concentration MBC) of ozonated water (0.6 ppm) were found to be in the range of 0.6-0.004 ppm for both A.a, and P.g. It can be

used at high dilution (low concentration) without losing its antimicrobial activity. On the other hand, the results showed that the MIC and MBC of Chlorhexidine were 62 ppm, and 250 ppm against A.a, and P.g respectively. The antimicrobial potency of Chlorhexidine depends mainly on the sensitivity of bacterial isolate.

The effect of contact times (1-15 minutes) of ozonated water and chlorhexidine on the number of bacterial colonies (A.a., and P.g.) on agar surface decreased gradually as the contact times increased from 1 to 15 minutes (negative correlation),and the colonies have been fully deactivated at 5 and 4 minutes respectively. While Chlorhexidine showed no effect against A.a up to 15 minutes, with complete deactivation in 10 minutes against P.g. The results verified that the P.g bacteria were much more sensitive toward ozonated water, and Chlorhexidine compared to A.a.

The effect of exposure time (1-10) minutes of ozonated air (218 ppm/W-Air) on the growth of colonies on the agar surface, showed that ozonated gas was effective on both A. a, and P.g colonies. After ozone exposure, the numbers of bacterial colonies on the agar surface decreased in a time-dependent manner and the colony's growth was no longer detected in 7, and 4 minutes of treatment against A. a and P.g. Respectively. The mean inhibition zone demonstrated by ozonated olive oil, ozonated water, and Chlorhexidine were 14.45 mm, 11.15 mm, and 9.653 mm respectively. This comparison revealed that there were highly significant differences between the antimicrobial agents. Overall, the results showed that ozonated olive oil was the most efficient antimicrobial agent followed by ozonated water and Chlorhexidine.

**Conclusions**: the antimicrobial activity of different forms of Ozone (aqueous, gaseous, and olive oil) were very efficient against A.a, and P.g isolates. These activities primarily depend on the contact, exposure times and the type of bacteria. The broad clinical application of ozone treatment in the periodontal disease seems to be a promising future.

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