

Comparative Evaluation Of Efficacy Of Ozonated Saline With Chlorhexidine Mouth Wash Prior To Non-Surgical Periodontal Intervention

^[1] Dr. Bakkiya. A, ^[2] Dr. Monisha. M, ^[3] Dr. Snophia Suresh, ^[4] Dr. Uma Sudhakar, ^[5] Dr. Dhatri Priya, ^[6] Dr. Navina Ravindran, ^[7] Dr. Lalitha Arunachalam, ^[8] Dr. Ajith Baskar

^{[1][2][8]} Post Graduate, Dept of Periodontics, Thaimoogambigai Dental College & Hospital, Chennai.

^{[3][7]} Professor, Dept of Periodontics, Thaimoogambigai Dental College & Hospital, Chennai

^[4] Professor & Head, Dept of Periodontics, Thaimoogambigai Dental College & Hospital, Chennai

^{[5][6]} Senior Lecturer, Dept of Periodontics, Thaimoogambigai Dental College & Hospital, Chennai.

To access & cite this article

Website: tmjpbs.com



ABSTRACT

Introduction: Ultrasonic Scaling is a potential source of aerosol contamination in dental clinics. The primary sources of microbial load in aerosols are the oral cavity and dental unit water line. Various antiseptic mouth rinses have been tried as preprocedural mouth rinsing to reduce the bacterial and viral load in the aerosol generated during ultrasonic scaling. Ozonated saline is a powerful antimicrobial agent against bacteria, fungi, and viruses, there is a paucity of literature regarding its preprocedural antimicrobial activity during ultrasonic scaling.

Aim: This study was aimed to determine the efficacy of ozonated saline as a preprocedural rinse in reducing bacterial load during ultrasonic scaling.

Materials and Methods: In this study, 45 subjects were selected who fulfilled the inclusion criteria and were divided into three groups. Group I subjects received saline as a preprocedural mouth rinse. Group II subjects received chlorhexidine as a preprocedural mouth rinse. Group III subjects received ozonated saline as a preprocedural mouth rinse. Aerosol produced during scaling was collected with a blood agar plate in the patient's chest, the clinician's right-hand, and two feet beside the patient. These plates were incubated at 37° C for 48 hours and Total Colony-Forming Units (TCFU) were counted.

Results: Ozonated saline showed effective TCFU reduction when compared to Group I Saline comparable with chlorhexidine.

Conclusion: Ozonated saline is more effective in reducing dental aerosols when compared to saline. Ozonated saline showed better TCFU reduction when compared with saline. Hence ozonated saline can also be used as a preprocedural mouth rinse for reducing the number of dental aerosols during ultrasonic scaling.

Key words: Nonsurgical periodontal therapy, ozonated water, chlorhexidine, colony-forming units.

INTRODUCTION

The oral environment provides an optimal medium for bacterial proliferation. The key contributors to aerosol production are the utilization of ultrasonic scaler and air rotor in the dental chair. These devices release tiny droplet nuclei particles that linger in the surrounding for extended duration and is a perilous factor for transmission of contagious diseases to the patient and the dental experts.¹ Within the dental community, infection control ranks among the foremost priorities. Infectious agents may be transmitted to patients as well as the dental personnel via several vectors, including instruments and air borne particles.²

When compared to manual scaling tools, the use of ultrasonic scaling is advantageous for removing dental deposits. Personal protective barriers, preprocedural rinses and high evacuation devices have been routinely used to reduce the aerosol in dental practice. Preprocedural rinsing is the norm for all aerosol generating procedures. Mouthwashes are made of a range of chemical substances and have the capacity to regulate the microorganism and reduce plaque accumulation.³ Chlorhexidine (CHX) stands as the predominant and widely accepted mouthwash, setting the benchmark standard. When used in low concentration, CHX functions as a bacteriostatic agent, whereas at high concentration exert a bactericidal effect. Nonetheless, CHX does present several drawbacks, encompassing the staining of teeth, impaired taste perception, supragingival calculus formation, and desquamation of oral mucosa.⁴

Apart from CHX, ozone possesses antiseptic properties and it acts against bacteria, fungi, and viruses. Ozone finds various applications in Dentistry, being utilized in diverse forms including its gaseous state applied through oils, as well as in aqueous form termed as ozonated water. It is well known that ozone, in the gaseous or aqueous phase, can kill bacteria, fungi, and viruses.⁵ Due to its inherent instability, ozone swiftly releases the nascent oxygen molecule results in oxygen gas formation. This characteristic has been used in human medicine to kill bacteria, fungus, inactivate viruses, and reduce haemorrhages because of its ability to liberate nascent oxygen.⁶ The advantages of ozone in the aqueous phase are its potency, ease of handling, lack of mutagenicity, rapid microbial effects, and suitability for use of the solution.⁷ While ozonated saline demonstrates potent antibacterial activities against various microorganisms, its effectiveness as an antimicrobial agent in the context of a

preprocedural mouth rinse prior to scaling procedure has not been extensively studied.

This present study is aimed to compare and evaluate the efficacy of ozonated saline as a preprocedural rinse with chlorhexidine mouth rinses prior to non-surgical periodontal therapy in reducing bacterial load during ultrasonic scaling.

MATERIALS AND METHODS:

Study population:

A study population including 45 subjects diagnosed with generalized chronic gingivitis were chosen from the Department of Periodontics at Thaimoogambigai Dental College & Hospital in Chennai. These subjects were matched based on their age and gender and were sorted into three groups.

Group I consisted of subjects who were administered saline solution as a preprocedural mouth rinse. In Group II, subjects received chlorhexidine as a preprocedural mouth rinse, while Group III subjects received ozonated water as a preprocedural mouth rinse. Prior to commencing the study, ethical clearance was acquired from the Institution Ethical Committee, and all the subjects provided their informed consent before participating.

Study design:

The dental operatory was sealed and underwent fumigation for duration of 18 hours as a preventive measure against contamination. Before the procedure commencement, a blood agar plate was kept in the fumigated chamber for 30 minutes as a standard reference. (Fig: 1) Subjects were comfortably seated in the dental chair. The subjects were instructed to rinse with 10 ml of mouthwash for duration of 1 minute. Aerosols produced during the scaling procedure were collected using blood agar plates positioned near the patient's chest, the clinician's right-hand, and two feet away from the subject. (Fig :2) Following incubation at 37°C for 48 hours, a microbiologist utilized a colony counter device to enumerate the Total Colony-Forming Units (TCFU) on these plates. The culture media used in this investigation was blood agar, a cost-effective and widely used for culturing bacterial colonies in laboratory settings.

Preparation of ozonated water:

Making ozonated water involves filling a cylinder with purified water while an ozone gas mixture

bubbles through it continuously.(Fig:3) This occurs for at least 15 minutes until maximum saturation has taken place. When ozone gas comes into contact with water, it becomes extremely unstable and reactive. As a result, a complicated sequence of chain reactions takes place, producing highly reactive hydroxyl (OH) radicals.

Statistical analysis:

The data of the present study was analyzed using IBM SPSS Statistics for Windows, Version 26.0 developed by IBM Corp., Armonk, NY. Descriptive statistics including mean, standard deviation, and standard error for a range of clinical parameters within the three distinct study groups. The examination of data normality through the Kolmogorov-Smirnov test revealed significant deviation from a normal distribution. As a result subsequent analysis employed a non-parametric test. Kruskal- Wallis test was employed, with adjustments made through the Bonferroni correction for pairwise comparisons, to evaluate the disparities in mean ranks among the three study groups. The established significance threshold in the present study was maintained at a level of $p < 0.05$.

RESULTS:

In this study, a total of 45 patients were chosen and subsequently allocated into three groups using the randomization process. These groups were categorized as Group I (saline), Group II (chlorhexidine), and Group III (ozonated water), with each group comprising 15 individuals.

Each group consisted of 15 subjects.

Table 1 illustrates the comparison of mean colony forming units among the three distinct groups at different locations. A statistically significant difference was noted.

Table 2 presents the results of the multiple pairwise comparison of the mean colony forming units between various groups. A statistical significant variation was observed when comparing the preprocedural rinsing of ozonated water and chlorhexidine with saline solution.

Discussion:

The transmission of infection to dental personnel and individuals within the dental operatory room through aerosols generated during the various dental

procedures has consistently remained a primary concern.⁸ Among various dental procedures, the ultrasonic scaling stands out for generating the highest volume of aerosols. While achieving complete elimination is challenging, adhering to protocols set forth by the American Dental Association, can lead to reduction in dental aerosols. To manage airborne contamination resulting from various dental procedures, plethora of techniques can be employed, including the use of barrier protection (mask, gloves, and eye protection), pre-procedural rinses with antiseptic mouthwash such as chlorhexidine, the utilization of high-volume evacuation systems, integration of high-efficiency particulate air room filters, and the application of ultraviolet treatment to the ventilation system. Several researchers have proposed that preprocedural rinsing represents the easiest and most efficient approach for minimizing the bacterial load within aerosols.

In this study, preprocedural mouth rinses were administered using chlorhexidine, ozonated water, and saline prior to scaling procedure in individuals having generalized chronic gingivitis. On comparison of mean colony forming units generated after scaling, comparable reduction was noted for chlorhexidine and ozonated water as preprocedural rinse. The antimicrobial efficacy of ozonated water is comparable to chlorhexidine in the present study. Chlorhexidine being a gold standard mouth rinse, exhibits a wide-ranging antimicrobial effect, targeting gram-positive and gram-negative bacteria, yeasts, dermatophytes and certain lipophilic viruses.⁹ Notably, it maintains its effectiveness for up to 12 hours, showcasing its substantivity. Muir et al. found that a preliminary 2 minute pre-rinse with CHX led to noteworthy reduction in aerosol generation produced by ultrasonic scalers, due to its extensive antimicrobial coverage and enduring impact, chlorhexidine is recognized as a gold standard for chemical plaque management.¹⁰ Logothetis & Martinez Welles, have shown that chlorhexidine preprocedural rinse was effective in reducing bacterial contamination with the use of an air polisher.¹¹ However contradictory results were obtained by Bay et al. who found rinsing immediately before air polishing with a 30 second pre-rinse of antiseptic mouthwash to be slightly more effective than chlorhexidine.¹²

In the present study, the culture plates were exposed to aerosols as per the protocol of Bentely et al to standardize the exposure time. Non selective culture media such as blood agar has been utilized in plethora

of studies.¹³ The aerobic organism settles over the agar plate and grows as colony is counted as colony forming units and their total number is counted.¹⁴

In the present study, aqueous ozone was freshly prepared each day by ozonation of distilled water for 20 minutes, by using an ozone generator. When compared to alternative chemical cleansing agents, ozonated saline has exhibited notable efficacy in combating bacteria, fungi, and viruses, with the added advantage being cost effective. When applied as a preprocedural mouth rinse for duration of 30 seconds, ozonated water has shown superior microbicidal potency than saline solution.¹⁵

Ozone is being employed across a wide spectrum of dental applications and is steadily gaining prevalence in daily dental practices.¹⁶ Ozone's application within dentistry serves as a highly effective substitute or complementary disinfectant to traditional antiseptics owing to its undeniable disinfection potential in comparison to other antiseptic agents.¹⁷ Hence ozonated water can be used as an alternative to chlorhexidine mouthwash prior to ultrasonic scaling.

The utilization of ozonated water could potentially contribute to the mitigation of oral infections and various microorganisms than volatilized ozone gas, which has been associated with adverse effect on the respiratory tract.¹⁸

Ozonated saline effectively eliminates gram-positive and gram-negative bacteria, as well as oral *Candida albicans*, associated with periodontal disease. Although rapid degradation is one of the major environmental advantages of ozonated water, this also produces a rapid decrease in microbicidal activity. The important factors in microbicidal activity are the quantity of ozone transferred to the water, contamination by dissolved organic compounds, temperature and pH.¹⁹ The aqueous form of ozone demonstrated less toxicity than volatilized gas or well recognised antimicrobials such chlorhexidine digluconate, sodium hypochlorite, or hydrogen peroxide.²⁰ This characteristic positions aqueous ozone as a biocompatible option for oral application, aligning with the cellular biology requirements for safe use.

Ozone, existing as a triatomic form of oxygen, is readily available in an active state and can be easily dispensed and administered in the clinical setting. Functioning as a extremely potent oxidizing agent, it

has showed to possess broad-spectrum antimicrobial activity. Hence the use of ozonated saline as a preprocedural mouth rinse has led to notable reduction in total colony –forming units.²¹

Conclusion:

Both chlorhexidine mouth rinses and ozonated saline exhibited a noticeable decline in Total Colony Counts when compared to saline. The outcomes of this current study highlight the comparable efficacy of ozonated mouthwash in reducing Total Colony-Forming Units. The advancement of ozone therapy should continue to focus on the establishing safe and precisely-defined parameters by randomized controlled trials and to explore the mechanisms involved and ensuring the enduring preservation of gingival and periodontal healing.

REFERENCES:

1. Veksler AE, Kayrouz GA, Newman MG. Reduction of salivary bacteria by pre-procedural rinses with chlorhexidine 0.12%. *J Periodontol.* 1991;62(11):649-651. doi:10.1902/jop.1991.62.11.649
2. Jawade R, Bhandari V, Ugale G, et al. Comparative Evaluation of Two Different Ultrasonic Liquid Coolants on Dental Aerosols. *J Clin Diagn Res.* 2016;10(7):ZC53-ZC57. doi:10.7860/JCDR/2016/20017.8173
3. Marcotte H, Lavoie MC. Oral microbial ecology and the role of salivary immunoglobulin A. *Microbiol Mol Biol Rev.* 1998;62(1):71-109. doi:10.1128/MMBR.62.1.71-109.1998
4. Runnells RR. An overview of infection control in dental practice. *J Prosthet Dent.* 1988;59(5):625-629. doi:10.1016/0022-3913(88)90083-2

5. Lu DP, Zambito RF. Aerosols and cross infection in dental practice--a historic view. *Gen Dent*. 1981;29(2):136-142.
6. Anumula L, Kumar KS, Krishna CM, Lakshmi KS. Antibacterial Activity of Freshly Prepared Ozonated Water and Chlorhexidine on Mutans Streptococcus When Used as an Oral Rinse - A Randomised Clinical Study. *J Clin Diagn Res*. 2017;11(7):ZC05-ZC08.
doi:10.7860/JCDR/2017/26708.10129
7. Gupta G, Mansi B. Ozone therapy in periodontics. *J Med Life*. 2012;5(1):59-67.
8. Holbrook WP, Muir KF, Macphee IT, Ross PW. Bacteriological investigation of the aerosol from ultrasonic scalers. *Br Dent J*. 1978;144(8):245-247. doi:10.1038/sj.bdj.4804072
9. Leggat PA, Kedjarune U. Bacterial aerosols in the dental clinic: a review. *Int Dent J*. 2001;51(1):39-44. doi:10.1002/j.1875-595x.2001.tb00816.x
10. Muir KF, Ross PW, MacPhee IT, Holbrook WP, Kowolik MJ. Reduction of microbial contamination from ultrasonic scalers. *Br Dent J*. 1978;145(3):76-78. doi:10.1038/sj.bdj.4804123
11. Logothetis DD, Martinez-Welles JM. Reducing bacterial aerosol contamination with a chlorhexidine gluconate pre-rinse. *J Am Dent Assoc*. 1995;126(12):1634-1639. doi:10.14219/jada.archive.1995.0111
12. Bay NL, Overman PR, Krust-Bray K, Cobb C, Gross KB. Effectiveness of antimicrobial mouthrinses on aerosols produced by an air polisher. *J Dent Hyg*. 1993;67(6):312-317.
13. Bentley CD, Burkhart NW, Crawford JJ. Evaluating spatter and aerosol contamination during dental procedures. *J Am Dent Assoc*. 1994;125(5):579-584.
doi:10.14219/jada.archive.1994.0093
14. Harrel SK, Molinari J. Aerosols and splatter in dentistry: a brief review of the literature and infection control implications. *J Am Dent Assoc*. 2004;135(4):429-437.
doi:10.14219/jada.archive.2004.0207
15. 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(3):341-348.
doi:10.4103/0970-9290.70796
16. Bonesvoll P, Lökken P, Rølla G, Paus PN. Retention of chlorhexidine in the human oral cavity after mouth rinses. *Arch Oral Biol*. 1974;19(3):209-212. doi:10.1016/0003-9969(74)90263-5
17. Staehelin J, Hoigne J. Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. *Environ Sci Technol*. 1985;19(12):1206-1213.

18. Nogales CG, Ferrari PH, Kantorovich EO, Lage-Marques JL. Ozone therapy in medicine and dentistry. *J Contemp Dent Pract.* 2008;9(4):75-84. Published 2008 May 1.

19. Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of literature. *J Dent.* 2008;36(2):104-116. doi:10.1016/j.jdent.2007.11.008

20. Huth KC, Jakob FM, Saugel B, et al. Effect of ozone on oral cells compared with established antimicrobials. *Eur J Oral Sci.* 2006;114(5):435-440. doi:10.1111/j.1600-0722.2006.00390.x

21. Talasani RR, Potharaju SP, Vijaya Lakshmi B, et al. Efficacy of ozonated water over chlorhexidine mouth rinse in chronic gingivitis patients - A comparative clinical study. *Saudi Dent J.* 2022;34(8):738-743. doi:10.1016/j.sdentj.2022.09.004

implantoplasty—A systematic review. *Clin Oral Impl Res.* 2019; 30: 833– 848.

26. Wohlfahrt JC, et al. Porous titanium granules in the surgical treatment of peri-implant osseous defects: a randomized clinical trial. *Int J Oral Maxillofac Implants.* 2012;27(2):401–10.

27. Zhang H, Li W, Zhang L, et al. A nomogram prediction of peri-implantitis in treated severe periodontitis patients: a 1-5-year prospective cohort

study. *Clin Implant Dent Relat Res.* 2018;20(6):962-968.

TABLES:

Table-1 Comparison of mean colony forming units with three mouth rinses in various Locations

Location	Mouthwash	N	Mean Rank	P- Value
CHRA	Saline	15	31.80	0.004
	Chlorhexidine	15	31.57	
	Ozonated water	15	32.47	
CHEST	Saline	15	20.90	0.008
	Chlorhexidine	15	18.37	
	Ozonated water	15	19.33	
2 Feet	Saline	15	16.30	0.003
	Chlorhexidine	15	19.07	

Table-2
Multiple
pairwise
comparison
of Colony
Forming
Units of 3
Groups in
various
Locations

Groups	Location	Test Statistic	Sig.
--------	----------	----------------	------

	Ozonated water	15	17.20	
TCFC	Saline	15	34.17	0.000
	Chlorhexidine	15	18.30	
	Ozonated water	15	16.53	
Ozonated water & CHX	CHRA	4.600	0.335	
Ozonated water & Saline		15.500	.001	
CHX & Saline		10.90	.002	
Ozonated water & CHX	Chest	-.700	.884	
Ozonated water & Saline		13.200	.006	
CHX & Saline		12.500	.009	
Ozonated water & CHX	2 Feet	-.700	.656	
Ozonated water & Saline		13.200	.001	
CHX & Saline		12.500	.006	
Ozonated water & CHX	TCFU	1.767	.712	
Ozonated water & Saline		17.633	.000	
CHX & Saline		15.867	.001	

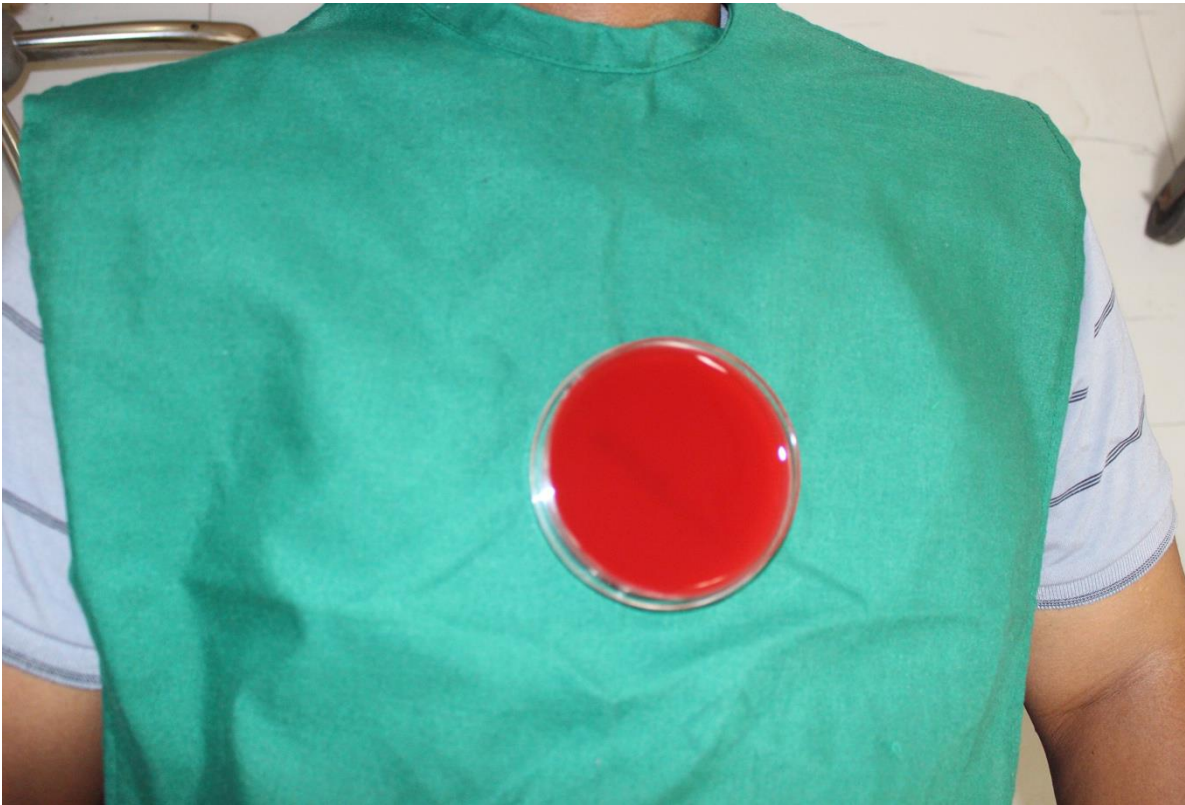


FIG :1 BLOOD AGAR PLATES



FIG: 2 COLONY FORMING UNITS



FIG:3 OZONATED WATER DISPENSER