Original Research Paper

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Ozonized Water as Pre-rinse and Chlorhexidine as Ultrasonic Coolant: A Savoir-faire in Times of Covid-19

Abstract:

Background: Use of high speed ultrasonics work under high pressure which generates spaltter and aerosols, contaminate the surroundings with various infectious agents. To reduce the infectious agents several procedures such as protective barriers, high volume evacuation device and prerinse. Ozonated water is antimicrobial and biocompatible making it suitable as pre-rinse dental use. Chlorhexidine is effective against broad spectrum bacteria attributing to its bicationic inhibitory action, bacterial cell protein chelation and high substantivity in the oral environment. This study aims to evaluate efficacy and microbial content of aerosols after prerinse with Ozonated water and 0.12% chlorhexidine as coolant in ultrasonic scalers.

Methodology: 40 participants were assigned to each group randomly Group A, Group B, Group C and Group D. Blood agar plates were placed on the chest of patients before the start of procedure.

Group A: pre rinse+ Chlorhexidine as coolant, **Group B:** pre rinse+ Distilled Water as coolant, **Group C:** Chlorhexidine as coolant

Group D: Distilled Water as coolant

After gravimetric settling, blood agar plates were transferred to the laboratory for incubation at 37°C for 48 h, followed by a colony-counting procedure by the microbiologist.

Results: Chlorhexidine as ultrasonic coolant with prerinse with ozonized water significantly reduces the microbial content of aerosols generated during scaling when compared with distilled water and without pre rinse.

Conclusion: Prerinsing with efficient antimicrobial mouthrinse before dental procedure minimizes the risk of infectious agent cross-contamination in the dental operatory.

Key-words: Ozonated water, antimicrobial mouthrinse, chlorhexidine, Ultrasonic coolant, aerosol

Introduction:

In recent years transmission of diseases to clinicians and cross contamination during numerous procedures has become a cause of increased concern.[1] The use of high speed airotors, ultrasonics, and air water syringes which work under high pressure, generates splatter and aerosols and contaminate the surroundings with various infectious agents.[2] Splatteris the larger liquid particles in the air having a size of more than 50 µm in diameter whereas aerosols are described as suspension of fine solid or liquid particles in the air having a size <50 µm in diameter.[3] The smaller particles are greatest potential risk because they invade and infect respiratory tract. SARS-COV-2 (Severe acute respiratory syndrome coronavirus 2) contaminated saliva of patients (either carrier or symptomatic) along with aerosols produced in the dental operatory, risks the entire dental employees working in the clinics.[4] Aerosol control in the dental setting is not an entirely new topic. Harrel and Molinari (2004) mentioned three principle levels of

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defense in handling aerosol as protective barriers, prerinse before scaling and use of high volume evacuation device, but the COVID-19 epidemic has obviously boosted interest in aerosol control methods and eventually necessitated droplet as well as airborne precautions.

Ozonated water is becoming an interested topic for its antibacterial and healing abilities, and its biocompatibility and disinfectant properties brought to light of research.[6] The effect of the ozonated water can be credited to the antimicrobial effects of ozone against periodontic microbes.[7] Hence, ozonated water is proved to be a good pre procedural rinse.[8] Ozone acts on bacterial cells by destructing Cytoplasmic membrane of bacterial cells affected due to ozonolys is of double bonds accompanied by oxidation of intracellular contents due to secondary oxidants effects.[6]

Chlorhexidine gluconate has been proven to be effective against broad spectrum bacteria because of its bacterial cell protein chelation, bi-cationic inhibitory action and long-time substantivity in the oral environment.[8,9] The cationic CHX is adsorbed in the cell membrane where it interacts with the anionic phosphate residue of the lipid molecules of bacterial cell membrane.[10]

In this pandemic era it is necessary to reduce the microbial contamination to bring down the cross infection in dental clinics to prevent patients and dentist from serious communicable diseases such as COVID-19 that transmits mainly through splatters and aerosols. So this study was conducted with the aim

1. To evaluate efficacy and microbial content of aerosols after pre- procedural rinse with Ozonated water.
2. To compare the potency and microbial content of aerosols of chlorhexidine as coolant in ultrasonic scalers.

Materials and Methods:

Inclusion criteria:

1. Systemically healthy patients having at least twenty permanent functional teeth.
2. No periodontal treatment within a 6 months of the study

Exclusion Criteria:

1. Use of tobacco in any form
2. Pregnant and lactating females
3. Patients under antibiotic or other drugs
4. Patients allergic to active ingredients used in study

Study design:

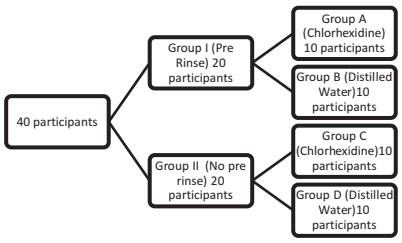


Figure 1: Study design

Procedure:

Subjects selected for this study were selected from outpatient in the Department of Periodontology. A total of 40 participants were included (male and female). Patients were informed about the procedures and a written consent was taken.

Patients were assigned to each group by the coin toss method (Group I or Group II) and again assigned to sub-groups by similar method (Group A, Group B, Group C and Group D) (Figure 1). Only one patient was treated per day, to avoid cross contamination. Before the treatment, the ultrasonic device was switched on and flushed out for 2 minutes, so as to remove contaminated water that has been collected in the water-pipes overnight. To minimize the contamination autoclaved mouth mask, head cap and disposable patient apron were used.

Blood agar plates were placed on the chest of patients before the start of procedure.(Figure 2)

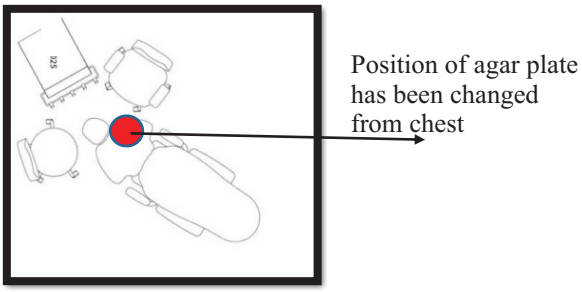


Figure 2: Position of Blood Agar Plate

Group I (Pre Rinse) 20 participants were asked to pre rinse with ozonated water for 30 seconds.[7] Ozonated water was made by using a tabletop "ozone generating device", by disolving ozone gas into distilled water for 1.6 minutes as advised by the manufacturer. Then participants were divided into subgroups as

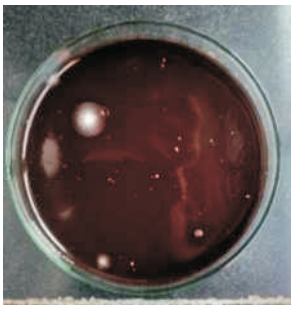
- Group A (0.12%Chlorhexidine as ultrasonic coolant)10 participants

- Group B (Distilled Water as ultrasonic coolant)10 participants

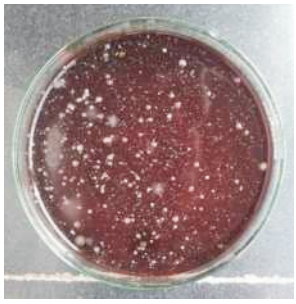
Group II (No pre rinse) 20 participants were not asked to pre rinses

- Group C (0.12%Chlorhexidine as ultrasonic coolant) 10 participants
- Group D (Distilled Water as ultrasonic coolant)10 participants

The ultrasonic scaling was performed for 20 min, with a universal tip connected to the ultrasonic scaler. The standard rate of flow of coolant in ultrasonic scaler is 20–30 ml/min in each group. Foreach procedure, suction was used. Post treatment, blood agar plates were left exposed for 20 min on center of dental chair for gravimetric settling of airborne pathogens. Then blood agar plates were sent to the laboratory for incubation at 37°C for 48 h, followed by a colony-counting technique by the microbiologist. (Figure 3 a, b, c, d). To prevent the inter-operator bias, all the patients were treated by a single operator.



a)Pre Rinse and Chlorhexidine coolant



b)Pre Rinse and Distilled Water coolant

c) No Pre Rinse and Chlorhexidine coolant

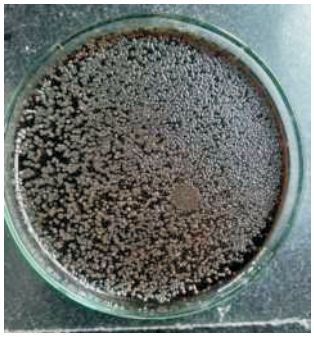


Figure 3: Bacterial colonies in agar plates in different groups.

Result:

Statistical analysis:

The Colony Forming Unit (CFU) data were summarized as Mean ± SE (standard error of the mean). The CFU data between two independent groups were compared by Student's t test. The CFU data between four independent groups were compared by one factor analysis of variance (ANOVA) and the significance of mean difference between (inter) the groups was done by Tukey's HSD (honestly significant difference) post hoc test after ascertaining normality by Shapiro-Wilk's test and homogeneity of variance between groups by Levene's test. The CFU data were analyzed on Log_{10} transformed data. A two-tailed (α=2) $P < 0.05$ was considered statistically significant. Analysis was performed on SPSS software (Windows version 22.0).

Results and Observations:

The CFU of two groups (pre rinse and no pre rinse) were summarized in Table 1 and also depicted in Figure. 4. The mean CFU of no pre rinse ranged from 4.0-7.0 with mean (± SE) 5.10 ± 0.16 and median 5 whereas in pre rinse it ranged from 0.7-5.0 with mean 3.00 ± 0.37 and median 4. The mean CFU was comparatively lower in pre rinse as compared to no pre rinse (pre rinse < no pre rinse).

Table 1: CFU (Mean ± SE, n=20) of two groups

No pre rinse(n=20)	Pre rinse (n=20)	Mean difference	t value	*P* value
5.10 ± 0.16	3.00 ± 0.37	2.10 ± 0.40	5.26	< 0.001

The CFU data were summarized in Mean ± SE and compared by Student's t test (t value).

Comparing the mean CFU of two groups, Student's t test showed significantly (P< 0.001) different and lower (41.2%) CFU in pre rinse as compared to no pre rinse.

The CFU of four groups (no pre rinse and DW coolant, no pre rinse and CHX coolant, pre rinse and DWcoolant, and pre rinse and CHX coolant) were summarised in Table 2 and also shown in Figure. 5. The mean CFU of pre rinse and CHX the least followed by pre rinse DW, no pre rinse and CHX, and no pre rinse and DW the maximum (pre rinse and CHX coolant< pre rinse and DW coolant< no pre rinse and CHX coolant< no pre rinse and DW coolant).

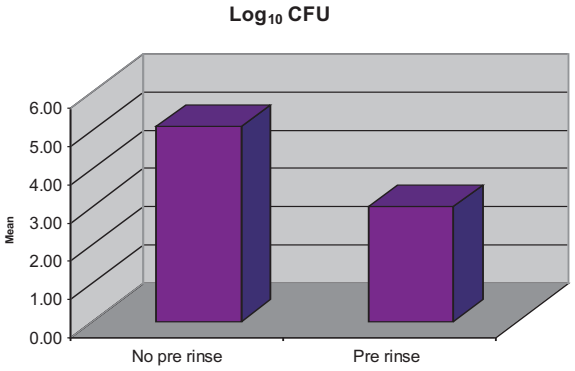


Figure 4. Mean CFU of two groups.

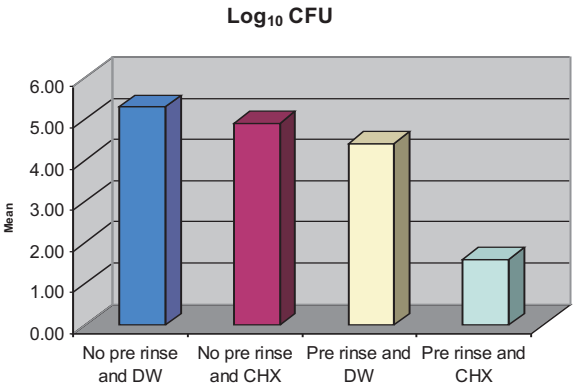


Fig. 5. Mean CFU of all four subgroups.

Comparing the mean CFU of four groups, ANOVA showed significantly different CFU among the groups (F=48.99, *P*< 0.001) (Table 3). Further, comparing the difference in mean CFU between the groups, Tukey test showed significantly (*P*< 0.001) different and lower CFU in pre rinse and CHX coolant group as compared to other three groups (no pre rinse and DW coolant, no pre rinse and CHX coolant, and pre rinse and DW coolant). However, it did not differ (*P*> 0.05) among no pre rinse and DW, no pre rinse and CHX, and pre rinse and DW i.e. found to be statistically the same.

Further, the mean CFU in pre rinse and CHX lower by 69.8, 67.4 and 63.7% as compared to no pre rinse and DW, no pre rinse and CHX, and pre rinse and DW respectively.

Table 2: CFU (Mean ± SE, n=10) of four groups

Group	CFU (Mean ± SE, n=10)	F value	*P* value
Group A: Pre rinse and CHX	1.60 ± 0.28	48.99	< 0.001
Group B:Pre rinse and DW	4.40 ± 0.22		
Group C:No pre rinse and CHX	4.90 ± 0.18		
Group D:No pre rinse and DW	5.30 ± 0.26		

The CFU data were summarized in Mean ± SE and compared by ANOVA (F value).

Table 3: Comparison of difference in mean CFU between groups by Tukey test

Comparison	Mean diff.	q value	*P* value	95% CI of diff.
No pre rinse and DW vs. No pre rinse and CHX	0.40	1.67	P > 0.05	-0.512 to 1.312
No pre rinse and DW vs. Pre rinse and DW	0.90	3.76	P > 0.05	-0.012 to 1.812
No pre rinse and DW vs. Pre rinse and CHX	3.70	15.47	P < 0.001	2.788 to 4.612
No pre rinse and CHX vs. Pre rinse and DW	0.50	2.09	P > 0.05	-0.412 to 1.412
No pre rinse and CHX vs. Pre rinse and CHX	3.30	13.80	P < 0.001	2.388 to 4.212
Pre rinse and DW vs. Pre rinse and CHX	2.80	11.71	P < 0.001	1.888 to 3.712

diff: difference, **q value**: Tukey test value, **CI**: confidence interval.

Discussion:

Periodontal diseases are associated with local factors of calculus and plaque and the host response.[11] Dental plaque is thought to be most important etiological factor in developing periodontal disease. Hence plaque removal is essential for treatment of gingival diseases. Conventional non-surgical therapy is considered as foundation of periodontal therapy that includes manual scaling and ultrasonic scaling.

Ultrasonic scalers work in principle of cavitation, microstreaming following generation of high frequency vibrations. However ultrasonic scalers produce aerosol and splatter. Aerosols are highly contaminated with microbes and can cause various health problems such as tuberculosis, severe acute respiratory syndrome (SARS), ophthalmic and skin infections. There are numerous methods for the protection of the dental personals to overcome the issue of blood-borne and airborne infections which includes use of 'universal precautions' such as, pre-procedural rinse, using high power suction, barrier methods, air filters in the operatory and using ultraviolet lights.[12]

The efficacy of pre-procedural rinsing has been proven in many studies.[13] CF Schonbein in 1839 discovered ozone and suggested it as a disinfectant for drinking water because

of its powerful ability to inactivate microorganisms (against bacteria, fungi, protozoa, and viruses).[14] Nagayoshi M concluded in his study that ozonated water combined with sonication, and used as an irrigant had nearly similar antimicrobial activity as 2.5% NaOCl, and exhibited a low level of toxicity against cultured cells.[15] Sadatullah S et al., measured the supragingival plaque microorganisms (total CFU) before and after rinsing once with 0.1 ppm of ozonated water and concluded that there was a reduction in the microbial load.[16] Ozonated water kills bacteria via neutralization and oxygenation of toxins produced by bacteria in oral cavitythere by reducing bacterial load in aerosol.

The length of ultrasonic scaler tip (4 and 7mm) and antimicrobial effect of pre-procedural rinsing does not spread to the depth of periodontal pocket.[17] Thus, there is a need for the use of an antimicrobial agent as a coolant to lessen the production of the contaminated aerosol generated. A study by Jawade al. has shown that CHX showed a superior reduction in CFU growth as compared to povidone-iodine.[18] CHX has proven its ability in controlling microorganism infection by aerosol contamination when it is used as coolant during ultrasonic scaling as it has antimicrobial activity that acts on inner cytoplasmic membrane.

This study was conducted to evaluate efficacy and microbial content of aerosols after pre-procedural rinse with ozonated water and to compare the potency and microbial content of aerosol of CHX as coolant in ultrasonic scaler.

Blood agar plates were used in this study to collect airborne microorganisms because it is considered as non-selective medium for culturing airborne bacteria. Aerosol is collected in plates and the bacteria present in aerosol is allowed to grow as colonies, it is considered as colony forming unit (CFU). This study revealed aerosol with high amount of bacteria is generated during ultrasonic scaling. The highest number of bacterial colonies were seen in the plates with no prerinse and DW group whereas prerinse & CHX group showed best result in preventing microorganism entrapment in aerosol. The use of CHX and or use of ozone water as prerinse, both showed positive result in reducing microorganisms in produced aerosol. However significant decrease in CFU was seen only in prerinse and CHX group when compared with all other three groups. The observations of this study could help to infer that prerinse with ozone water followed by scaling under CHX as coolant reduced significant retrievable CFU counts.

Conclusion:

This study indicates that CHX gluconate used as an ultrasonic liquid coolant with preprocedural mouthrinse with ozonized water significantly reduces the microbial load in aerosols generated during scaling as compared to distilled water and without pre rinse. Hence, CHX can be used as an ultrasonic coolant for diminishing the bacterial load of aerosols during ultrasonic scaling. This study also showed that Ozonated water rinses were found to be effective in reducing aerosol bacteria. Pre-procedural rinsing with an effective antimicrobial mouthrinse combined with an antimicrobial agent as coolant during any aerosol generating dental procedure diminishes the risk of infectious agent cross-contamination in the dental clinics.

Limitations:

The current study includes only aerobic bacteria that are capable to grow on blood agar plates. Anaerobic bacteria and viruses that needs specialized culture process, were not investigated, so this study does not differentiate these bacteria based on culture characteristics.

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