

Efficacy of adjunctive usage of hydrogen peroxide with chlorhexidine as preprocedural mouthrinse on dental aerosol

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ABSTRACT

Background: The microbial reservoirs of infectious bioaerosols include saliva, calculus, and dental unit water line. Previous studies have observed a reduction in salivary bacterial counts with the usage of chlorhexidine. In this study, calculus has additionally been targeted with the adjunctive usage of hydrogen peroxide followed by chlorhexidine mouthrinse. **Aims and Objective:** To evaluate the efficacy of preconditioning using 1.5% hydrogen peroxide followed by rinsing with 0.2% chlorhexidine over chlorhexidine alone, with saline as a negative control in reducing the microbial counts in the aerosol produced during ultrasonic scaling. **Methods:** Fifteen chronic periodontitis subjects were randomly allocated into three groups. Before scaling, groups 1 and 2 subjects were made to rinse with saline and 0.2% chlorhexidine, respectively. In group 3 patients, calculus was conditioned with a topical application of 1.5% hydrogen peroxide before chlorhexidine mouthrinse. The aerosol produced from the ultrasonic unit was collected at three designated areas on agar plates. The plates were incubated at 37°C for 48 h and colony-forming units (CFU) evaluated. **Result:** The CFUs were the lowest in group 3 at the three designated locations ($p = 0.007, 0.006, \text{ and } 0.003$, respectively). Hydrogen peroxide as an adjunct to chlorhexidine showed a statistically significant decrease in the CFUs at the patient and assistant sides, when compared with chlorhexidine alone. **Conclusion:** This pilot study showed that the adjunctive use of hydrogen peroxide with chlorhexidine is superior to lone usage of chlorhexidine in combating the infected dental aerosols.

KEY WORDS: Chlorhexidine; Hydrogen Peroxide; Dental Aerosol; Preprocedural Mouthrinse


INTRODUCTION

The dental personnel and the patients in a dental office can acquire infections because of the aerosols and splatter produced during the dental procedures. The plethora of cross infections is tuberculosis,^[1] hepatitis B,^[2] and respiratory

infections.^[3] Ultrasonic scalers, dental handpieces, air polishers, and air abrasion units produce the most visible and viable bioaerosols. Araujo and Andreana^[4] demonstrated that the ultrasonic scaler is the major source of potential aerosol contamination in a dental setup.

The components of the dental aerosol may vary according to each patient, and it can include saliva, nasopharyngeal secretions, plaque/calculus, blood, tooth components, and any dental material, such as abrasives for air polishing, used during the procedure.^[5] It is reasonable to suppose that the reduction of bacteria in these components can deteriorate the infectious potential of the aerosols.

Owing to its robust antibacterial property and substantivity, chlorhexidine remains the gold standard among the mouth rinses. Various studies have corroborated the superiority of

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chlorhexidine as a preprocedural mouthrinse over quaternary ammonium compounds and herbal extracts in reducing the viability of the bioaerosol.

Hydrogen peroxide is an oxidizer that has been employed in plaque control. Application of oxygenating agents include in the controlling of supragingival plaque and in the treatment of acute ulcerative gingivitis with no potential side effects to the tissues.^[6] A study demonstrated that the usage of 1.5% hydrogen peroxide mouthwash as an adjunct to chlorhexidine proved to be a better antiplaque agent when compared with chlorhexidine alone.^[7] It was attributed to the additive effect as both the chemicals have different modes of action on the bacteria. The antiplaque action of chlorhexidine is purely on the surface. In 1992, Seymour and Heasman^[8] stated that killing of bacterial cells is initially dependent on the drug having access to cell walls. Hydrogen peroxide acts therapeutically by releasing oxygen that immediately kills the obligate anaerobes present in the oral infections.^[9]

Hence, in this pilot study, the efficacy of preconditioning using 1.5% hydrogen peroxide followed by rinsing with 0.2% chlorhexidine was evaluated over chlorhexidine alone, with saline as a negative control in reducing the microbial counts in the aerosol produced during ultrasonic scaling.

MATERIALS AND METHODS:

This pilot study, which was a single-blinded randomized-controlled trial, was conducted on outpatients of the Department of Periodontics, Saveetha Dental College and Hospital, Tamil Nadu, India. The study protocol was approved by the Ethical Committee of the Institutional Review Board (IHEC/SDMDS13PER4). A total of 15 chronic periodontitis subjects which included eight men and seven women were recruited for this pilot study. Patient's age ranged from 35 to 50 years, with a mean age of 40 years. All the study participants were informed of the purpose, protocol, and the duration of the study. An informed consent was obtained from eligible candidates who were willing to participate.

Inclusion Criteria:

Chronic periodontitis subjects with a minimum of 20 permanent teeth and having clinical attachment loss of >3 mm in more than 30% of the sites were included. They were systemically healthy with plaque and calculus component score of ≥ 2 in the oral hygiene index.

Exclusion Criteria:

1. Topical/systemic antibiotic usage in the last 3 months
2. Oral prophylaxis within the last 3 months
3. Regular usage of mouthrinses
4. Smokers
5. Allergic to chlorhexidine/hydrogen peroxide
6. Pregnant/lactating women.

Materials:

1. 0.2% chlorhexidine gluconate
2. 1.5% hydrogen peroxide
3. Normal saline
4. Nutrient agar
5. Disposable Petri dishes (100 mm)
6. Applicator tip.

Grouping:

1. Group 1 subjects: 15 mL of normal saline for 2 min.
2. Group 2 subjects: 15 mL of 0.2% chlorhexidine gluconate for 2 min.
3. Group 3 subjects: Preconditioning with 1.5% hydrogen peroxide for 1 min followed by rinsing with 15 mL of 0.2% chlorhexidine gluconate for 2 min.

Study Design:

The selected subjects were assigned to one of the three treatment groups using numbered paper lots. Ten minutes before performing ultrasonic scaling, group 1 patients were instructed to rinse with 15 mL of saline for 2 min. Group 2 subjects were asked to rinse using 15 mL of 0.2% chlorhexidine gluconate (Hexidine[®]) for 2 min. In group 3 patients, preconditioning of the calculus deposits was done with 1.5% hydrogen peroxide (Hydrogen peroxide solution IP[®]—20 volume) using an applicator tip. The desired concentration of hydrogen peroxide was obtained by mixing one part with four parts of distilled water. After 1 min of conditioning, the patients were asked to rinse with 15 mL of 0.2% chlorhexidine (Hexidine) for 2 min.

A closed operatory room with minimal cross ventilation was chosen for all the treatment procedures. The predesignated agar plates (100 mm) were left uncovered and placed at standardized positions, i.e., patient's chest, doctor's side, and assistant's side. At the patient's chest area, the plate was positioned approximately 10 in. from the patient's mouth, whereas it was placed at a distance of 2 ft at the operator and assistant sides. The operator performed the ultrasonic scaling for a standardized time period of 5 min on a dental chair under controlled frequency and water pressure. A piezoelectric scaler unit and a high vacuum suction were used to perform the oral prophylaxis. Before each prophylaxis, the water from the scaler unit was flushed out for 30 s in order to reduce the microbial accumulation owing to water stagnation in the dental unit waterline.

The collected samples were transported to the Department of Microbiology, Saveetha Dental College, where it was incubated at 37°C for 48 h. The treatment groups were masked from the microbiologist, who in turn performed the counting of the number of colony-forming units (CFUs) that grew on each plate.

Statistical Analysis:

Statistical tests were performed using the SPSS Macros software. The values obtained were subjected to normality tests such as

Table 1: Kruskal–Wallis test for comparison of CFU at different sites

| Variable | Mouthrinse | Sample size | Mean rank | <i>p</i> |
|-----------------------|-------------------------------------|-------------|-----------|----------|
| CFU at operator side | Saline | 5 | 12.80 | 0.007 |
| | Chlorhexidine | 5 | 7.20 | |
| | CHX + H ₂ O ₂ | 5 | 4.00 | |
| CFU at patient side | Saline | 5 | 12.60 | 0.003 |
| | Chlorhexidine | 5 | 8.40 | |
| | CHX + H ₂ O ₂ | 5 | 3.00 | |
| CFU at assistant side | Saline | 5 | 11.80 | 0.006 |
| | Chlorhexidine | 5 | 9.20 | |
| | CHX + H ₂ O ₂ | 5 | 3.00 | |

p < 0.05 (significant).

CFU, colony-forming units; CHX, chlorhexidine; H₂O₂, hydrogen peroxide.

Kolmogorov–Smirnov and Shapiro–Wilk’s tests, and the resultant data showed that they followed a nonparametric distribution.

Hence, the overall comparison between the groups was obtained with Kruskal–Wallis test, and further pairwise comparisons were derived with Mann–Whitney test. Values of *p* < 0.05 were considered as statistically significant.

RESULTS

For evaluation of the antibacterial efficacy of the preprocedural mouth rinses, samples from 15 patients were statistically analyzed. On comparing the three locations, the microbial counts were highest at the patient’s chest area, followed by the operator and the assistant sides.

Among the groups, the number of CFUs at each location, i.e., patient’s chest, operator’s side, and assistant’s side, was significantly reduced in group 3 subjects who were treated with a topical application of 1.5% hydrogen peroxide as an adjunct to 0.2% chlorhexidine preprocedural mouth rinse. This has been illustrated in Table 1.

Table 2: Mann–Whitney test with Bonferroni correction for pairwise comparison of CFU at patient side

| Variable | Mouthrinse | Sample size | Mean rank | <i>p</i> |
|---------------------|-------------------------------------|-------------|-----------|----------|
| CFU at patient side | Saline | 5 | 7.60 | 0.032 |
| | Chlorhexidine | 5 | 3.40 | |
| CFU at patient side | Saline | 5 | 8.00 | 0.008 |
| | CHX + H ₂ O ₂ | 5 | 3.00 | |
| CFU at patient side | Chlorhexidine | 5 | 8.00 | 0.008 |
| | CHX + H ₂ O ₂ | 5 | 3.00 | |

p < 0.05 (significant).

CFU, colony-forming units; CHX, chlorhexidine; H₂O₂, hydrogen peroxide.

Table 3: Mann–Whitney test with Bonferroni correction for pairwise comparison of CFU at assistant side

| Variable | Mouthrinse | Sample size | Mean rank | <i>p</i> |
|-----------------------|-------------------------------------|-------------|-----------|----------|
| CFU at assistant side | Saline | 5 | 6.80 | 0.022 |
| | Chlorhexidine | 5 | 4.20 | |
| CFU at assistant side | Saline | 5 | 8.00 | 0.008 |
| | CHX + H ₂ O ₂ | 5 | 3.00 | |
| CFU at assistant side | Chlorhexidine | 5 | 8.00 | 0.008 |
| | CHX + H ₂ O ₂ | 5 | 3.00 | |

p < 0.05 (significant).

CFU, colony-forming units; CHX, chlorhexidine; H₂O₂, hydrogen peroxide.

A pairwise comparison revealed that the number of CFUs was the lowest in group 3, i.e., topical application of hydrogen peroxide followed by chlorhexidine prerinse when compared with the lone usage of chlorhexidine and saline. This statistical significance was obtained at the patient’s chest and assistant’s side [Tables 2 and 3]. At the operator’s side, statistical significance could not be obtained even though the number of CFUs was the lowest in group 3 subjects. These results have been depicted in Table 4.

DISCUSSION

Infection control has become a key component in a dental operatory, and various guidelines have been proposed to ensure the utmost safety of the patients and the dental personnel. Universal precautions were recommended by the Centers for Disease Control (CDC) to be used on all patients.^[10] An updated version of the standard and transmission-based precautions for dental health care personnel (DHCP) has also been put forward in order to keep the DHCP aware of the infection control programs and additional measures necessary to take when treating patients in their offices who are actively infected with certain organisms.^[11]

Table 4: Mann–Whitney test with Bonferroni correction for pairwise comparison of CFU at operator side

| Variable | Mouthrinse | Sample size | Mean rank | <i>P</i> |
|----------------------|-------------------------------------|-------------|-----------|----------|
| CFU at operator side | Saline | 5 | 7.80 | 0.016 |
| | Chlorhexidine | 5 | 3.20 | |
| CFU at operator side | Saline | 5 | 8.00 | 0.008 |
| | CHX + H ₂ O ₂ | 5 | 3.00 | |
| CFU at operator side | Chlorhexidine | 5 | 7.00 | 0.151 |
| | CHX + H ₂ O ₂ | 5 | 4.00 | |

p < 0.05 (significant).

CFU, colony-forming units; CHX, chlorhexidine; H₂O₂, hydrogen peroxide.

The bacterial aerosols are a constant threat to the dental fraternity, and their complete elimination still remains a daunting challenge. Aerosols can get contaminated by the microbes harboring in saliva, plaque/calculus, blood, tooth component, dental unit water line, and so on. The use of antiseptic preprocedural mouth rinses remains as one of the means to reduce the microbial load. Chlorhexidine takes the crown among them with various *in vitro* and *in vivo* studies showing a reduction in vital salivary bacteria,^[12,13] which is one among the reservoirs for contaminated aerosols. Hydrogen peroxide has an antiplaque potential because of its ability to produce free radicals. The stigma to its usage is due to side effects such as irritation, sloughing of tissues. So, in our study, a topical application of the ideal concentration of hydrogen peroxide (1.5%) to be used in the oral cavity was preferred over the mouthrinse form of the same concentration.

In this pilot study, the patient's chest area was exposed to a greater number of microorganisms, followed by the operator and the assistant sides. This is in accordance with the study conducted by Gupta *et al.* in 2013,^[14] which reinforces the necessity for personal barrier equipment such as face and eye shields, mask, head cap, gloves, and gown. The obtained results followed a nonparametric distribution that can be attributed to the fact that number of CFUs is highly variable among individuals and does not follow a consistent pattern.

A study by Swaminathan *et al.*, in 2013,^[15] observed the microbial counts in saliva and aerosol. They showed that there was 99.91% reduction in the salivary microbial load, before and after the usage of chlorhexidine as a preprocedural mouth rinse. It was also observed that it did not translate into a proportionate reduction of the aerosol load in the same group. In concordance, our study shows that the adjunctive usage of hydrogen peroxide with chlorhexidine is much more efficacious in reducing the aerosol load than chlorhexidine alone, because two of the microbial reservoirs have been targeted. It can be assumed that the salivary microorganisms are susceptible to chlorhexidine and the calculus component is acted upon by hydrogen peroxide, which also facilitates a deeper penetrant action of chlorhexidine, which, in turn, causes depreciation in the aerosol microbial counts.

The future perspective of this pilot study can be targeted toward performing clinical trials with a larger sample size in order to corroborate the results obtained from this study. Moreover, anaerobic organisms, viruses, and other organisms, which require specialized growth media, can be evaluated in the future trials.

Thus, within the limitations of this study, it can be concluded that the adjunctive usage of 1.5% hydrogen peroxide with 0.2% chlorhexidine is more efficacious and beneficial in reducing the microbial load in the aerosol produced during ultrasonic scaling when compared with the lone usage of chlorhexidine.

CONCLUSION

This pilot study marks the first step in establishing the superiority of hydrogen peroxide with chlorhexidine over the lone usage of chlorhexidine in reducing the aerosol contamination produced during scaling. Future studies with a larger sample size can be directed toward validating the evidence obtained from this study.

A strong emphasis toward the usage of preprocedural mouth rinses and infection control protocols are reestablished, and dental health-care personnel are urged to follow the same in order to reap the protective benefits of preventing cross-infection to the patients.

REFERENCES

1. Shaw AB. Tuberculosis in medical and dental students; a study at Guy's hospital. *Lancet*. 1952;2(6731):400-4.
2. Goldman HS, Hartman KS. Infectious diseases. Their disease, our unease: infectious diseases and dental practice. *Va Dent J*. 1986;63(2):10-9.
3. Miller RL. Generation of airborne infection ... by high speed dental equipment. *J Am Soc Prev Dent*. 1976;6(3):14-7.
4. Araujo MW, Andreana S. Risk and prevention of transmission of infectious diseases in dentistry. *Quintessence Int*. 2002;33:376-82.
5. 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):429-37.
6. Dona BL, Gründemann LJ, Steinfors J, Timmerman MF, van der Weijden GA. The inhibitory effect of combining chlorhexidine and hydrogen peroxide on 3-day plaque accumulation. *J Clin Periodontol* 1998;25(11 Pt 1) 879-83.
7. Jhingta P, Bhardwaj A, Sharma D, Kumar N, Bhardwaj VK, Vaid S. Effect of hydrogen peroxide mouthwash as an adjunct to chlorhexidine on stains and plaque. *J Indian Soc Periodontol*. 2013;17(4):449-53.
8. Seymour RA, Heasman PA. Anti-plaque and anti-calculus agents. In: *Drugs, Diseases, and the Periodontium*. New York: Oxford University Press, 1992. pp. 153-79.
9. Moran J, Addy M, Wade W, Milson S, McAndrew R, Newcombe RG. The effect of oxidising mouthrinses compared with chlorhexidine on salivary bacterial counts and plaque regrowth. *J Clin Periodontol*. 1995;22(10):750-5.
10. Centers for Disease Control (CDC). Recommendations for preventing transmission of infection with human T-lymphotropic virus type III/lymphadenopathy-associated virus in the workplace *MMWR Morb Mortal Wkly Rep*. 1985;34(45):681-6, 691-5.
11. Harte JA. Standard and transmission-based precautions: an update for dentistry. *J Am Dent Assoc*. 2010;141(5):572-81.
12. Da Silva NB, Alexandria AK, De Lima AL, Claudino LV, De Oliveira Carneiro TF, Da Costa AC, *et al.* *In vitro* antimicrobial activity of mouth washes and herbal products against dental biofilm-forming bacteria. *Contemp Clin Dent*. 2012;3(3):302-5.

13. Wikén Albertsson K, Persson A, van Dijken JW. Effect of essential oils containing and alcohol-free chlorhexidine mouthrinses on cariogenic micro-organisms in human saliva. *Acta Odontol Scand.* 2013;71(3-4):883-91.
14. Gupta G, Mitra D, Ashok KP, Gupta A, Soni S, Ahmed S, et al. Efficacy of preprocedural mouth rinsing in reducing aerosol contamination produced by ultrasonic scaler: a pilot study. *J Periodontol.* 2014;85(4):562-8.
15. Swaminathan Y, Thomas JT, Muralidharan NP. The efficacy of preprocedural mouth rinse of 0.2% chlorhexidine and commercially available herbal mouth containing *Salvadora persica* in

reducing the bacterial load in saliva and aerosol produced during scaling. *Asian J Pharm Clin Res.* 2014;7(Suppl 1):71-4.

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