Comparative Evaluation of the Effect of 0.2% Chlorhexidine, 3% Hydrogen Peroxide with Blue light and Blue light alone as agents to reduce aerolized bacteria- A Randomized Controlled Clinical Trial

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Abstract:

Background: Dental aerosol-generating procedures cerate's a significant volume of splatters and aerosols that raise serious concerns about the spread of airborne diseases in the dental operatory. Although barrier techniques have been utilized for a long time, cross-infection remains a clinical concern. Therefore, it was constantly needed to develop a novel method to lower the bioaerosols created by diverse dental equipment.

Aim: Evaluate the effect of 0.2% Chlorhexidine, 3% Hydrogen Peroxide with Blue light and Blue light alone as agents to reduce aerolized bacteria

Material and Methods: Sixty patients were randomly categorized into three groups. Group I patients were asked to rinse their oral cavity with 10ml of 0.2% Chlorhexidine for a minute followed by irradiation with blue light for 1 minute. Group II patients rinsed the oral cavity with 10ml of 3% Hydrogen Peroxide followed by 1 minute of irradiation with blue light. In Group III patients oral cavity was only exposed to the blue light irradiation for 1 minute. All the patients underwent ultrasonic scaling for 30 minutes. Prior to the starting of ultrasonic scaling blood agar plate was placed on the patient's chest area. Upon completion of Ultrasonic scaling for 30 minutes the blood agar plate was evaluated for CFUs. Data was obtained and tabulated followed by Stastical analysis using SPSS (Statistical Package for Social Sciences) 25.0 version.

Results: The subjects of group 2 (H_2 O_2 + blue light) harbored significantly ($p<.05$) lower CFUs than group 1 (0.2% Chlorhexidine) and group 3 (Blue light). The count of colony-forming units was significantly large in group 3 compared to groups 1 and 2. The count of colony-forming units was significantly large in group 1 compared to group 2 but significantly less than in group 3 (p-value <.05).

Conclusion: The formation of bioaerosols was shown to be significantly reduced by 3% H_2 O_2 and LED Blue light as compared to the commonly used antibacterial 0.2 Chlorhexidine or Blue light.

Key Words: Aerosols, bioaerosols, Blue light, 0.2% Chlorhexidine, Dental aerosol, 3% Hydrogen Peroxide, Photodynamic therapy (PDT), Pre-procedural rinse

Introduction:

Dental office plays an important contributing factor in production of bioaerosols which is an important occupational hazard placing dental professionals, staff and patients at an increased risk of contracting airborne disease. Various dental instruments and equipment like ultrasonic devices, high speed hand pieces and three way syringes used in various oral surgical procedures can generate and propagate these bioaerosols.[1]

Aerosols can be defined as particles suspended in gas and either anthropogenic (produced by humans or animals by coughing) or natural (such as fog, dust), sneezing or by mere speaking, whereas bioaerosols contain live microorganisms within the aerosols. [2]

Prevention of airborne infections by limiting or reducing these bioaerosols in dental office through various materials, techniques and strategies has been practiced over years. These include pre-procedural rinse with anti-microbial agents, use of rubber dam during, high volume evacuators, general cross ventilation of the clinics and use of HEPA filters.[3]

This especially is very important with emergence of new airborne diseases like COVID-19 and more so in a multichair dental clinics. Hence a constant search for new strategies and techniques are the need of the hour that is quite effective and also economical.

Pre-procedural rinses using antimicrobial agents prior to any oral non-surgical or surgical procedure has been one of the cost effective and safe technique that have been practiced to limit the bacterial load in the oral cavity or in the aerosols produced. A variety of antimicrobial or antiseptic rinses have been reported in the literature to reduce the microbial or viral transmission via bioaerosol production. Oral rinses like Chlorhexidine, Povidone-Iodine (PVP-I), Essential oils, Cetylpyridinium chloride (CPC), Hydrogen Peroxide, Herbal preparations have been used with varying success.[4,5]

Photodynamic therapy (PDT) is a novel therapeutic treatment protocol which is used as an alternative to chemical antimicrobial agents to eliminate periodontopathogenic microorganism.[6] Photodynamic therapy involves Visible light, Photosensitizer and Oxygen. Quartz-tungsten-halogen lamps that irradiate blue light (400–520 nm wavelengths) are effective in eradicating pathogenic microorganisms. [7,8]

Not many Studies exist in periodontal literature evaluating the effect of Hydrogen Peroxide with blue light and Blue light alone as a substitute for conventionally used preprocedural rinses. Hence this study was designed and aimed to evaluate the effect of 0.2% Chlorhexidine, 3% Hydrogen Peroxide with Blue light and Blue light alone as agents to reduce aerolized bacteria.

Material and Methods

The present study was single center, double blind, placebo controlled, randomized, three group parallel arm clinical trial. The study was approved by the Institutional Ethics Committee (IIDS/IEC/2022/177(E)/PERIO/03). Sixty systemically healthy patients reporting the outpatient section, Department of Periodontology, Index Institute of Dental Sciences, Indore in the age group of 30-55 years diagnosed with Stage I Grade A Periodontitis (>30% of sites with PPD ≤5mm) and fulfilling the following criteria were selected for the study. Patients having a minimum of 20 teeth, not received any periodontal treatment during last 6 months and are not using oral mouth washes as part of their regular oral hygiene measures. The study population was randomly divided into 3 distinct patient groups.

Group I consisted of patients who used 10ml of 0.2% Chlorhexidine as pre-procedural rinse for 1 minute (n=20), Group II Patients rinsed their oral cavity with 10ml of 3% Hydrogen Peroxide for 1 minute followed by irradiation with blue light for 1 minute. In Group III oral cavity was only exposed to the blue light irradiation for 1 minute.

Medical and dental history was obtained followed by a full mouth clinical periodontal examination like Plaque Index (PI), Gingival Index (GI), Pocket depth (PD) and Clinical attachment loss (CAL) was recorded using William's Periodontal Probe for all the patients prior to the procedure. All the patients underwent ultrasonic scaling for 30 minutes. Prior to the starting of ultrasonic scaling Blood agar plate was placed on the patient's chest area. Upon completion of Ultrasonic scaling for 30 minutes the blood agar plate was sent to the Department of Biochemistry, Index Medical College and Hospital, Indore for analysis of CFU.

Statistical Analysis:

Data were entered into the Excel sheet. Data were analyzed using SPSS (Statistical Package for Social Sciences) 25.0 version, IBM, Chicago. Data were analyzed for probability distribution using the Kolmogorov-Smirnov test and was found to be not normally distributed and thus, non-parametric tests of significance were applied. Descriptive statistics were performed. The inter-group comparison was done using the Kruskal-Wallis test. A p-value < .05 was considered statistically significant.

Results

The clinical parameters GI, PI, PD and CAL of the study subjects are interpreted in Table1and 2 and Figure 1 and 2. There was no significant difference in plaque and gingival scores among subjects belonging to the three groups (p>.05). Likewise there was also no significant difference in the probing depths and clinical attachment levels between the subjects belonging to the three groups (p>.05).

Comparing the three groups with respect to CFUs, the subjects of group 2 (H_2O_2 + blue light) harbored significantly (p<.05) lower CFUs than group 1 (0.2% Chlorhexidine) and group 3 (Blue light). Table 3 and Figure 3 shows the comparison of CFUs in group 1, group 2, group 3. Results reveal that bioaerosols contamination was significantly lower in subjects of group 2 compared to subjects of group 1, followed by subjects of group 3 .

Post hoc analysis (CFU) revealed that count of CFUs was largely in group 3 (Figure 4) compared to group 1(Figure 5) and 2 (Figure 6). The count of CFU was significantly large in group 1 compared to group 2 but significantly less than in group 3 (p-value <0.5).

Table 1. Comparison of plaque index and gingival index.

	Group 1		Group 2		Group 3		Chi-square value	P-value[a]
	Median	IQR	Median	IQR	Median	IQR		

Plaque index	2.0	1.5-2.0	1.75	1.5-2.0	2.0	1.625-2.0	4.055	.132
Gingival index	2.0	1.5-2.0	2.0	1.5-2.0	2.0	2.0-2.0	4.549	.103

IQR- Inter-quartile range. [a]Kruskal-Wallis test

Table 2. PPD and CAL amongst 3 groups.

	Group 1		Group 2		Group 3		Chi-square value	P-value[a]
	Median	IQR	Median	IQR	Median	IQR		
Periodontal pocket depth	5.0	4.0-5.0	5.0	4.0-5.0	4.0	4.0-5.0	2.158	.340
Clinical attachment loss	2.0	2.0-3.0	2.0	1.25-3.0	2.0	2.0-2.0	.054	.973

IQR- Inter-quartile range. [a]Kruskal-Wallis test

Figure 1. Plaque index score and gingival index score amongst 3 groups.

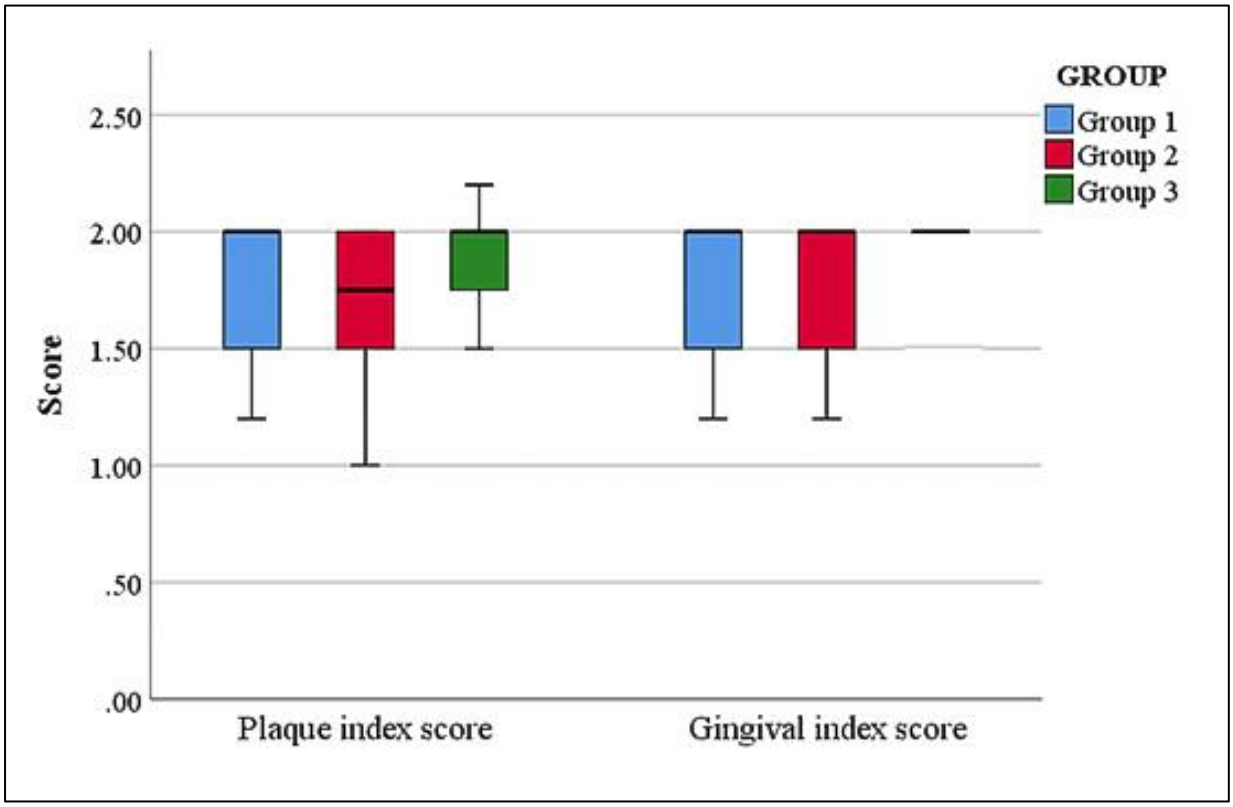

Figure 2. Comparison of PPD and CAL.

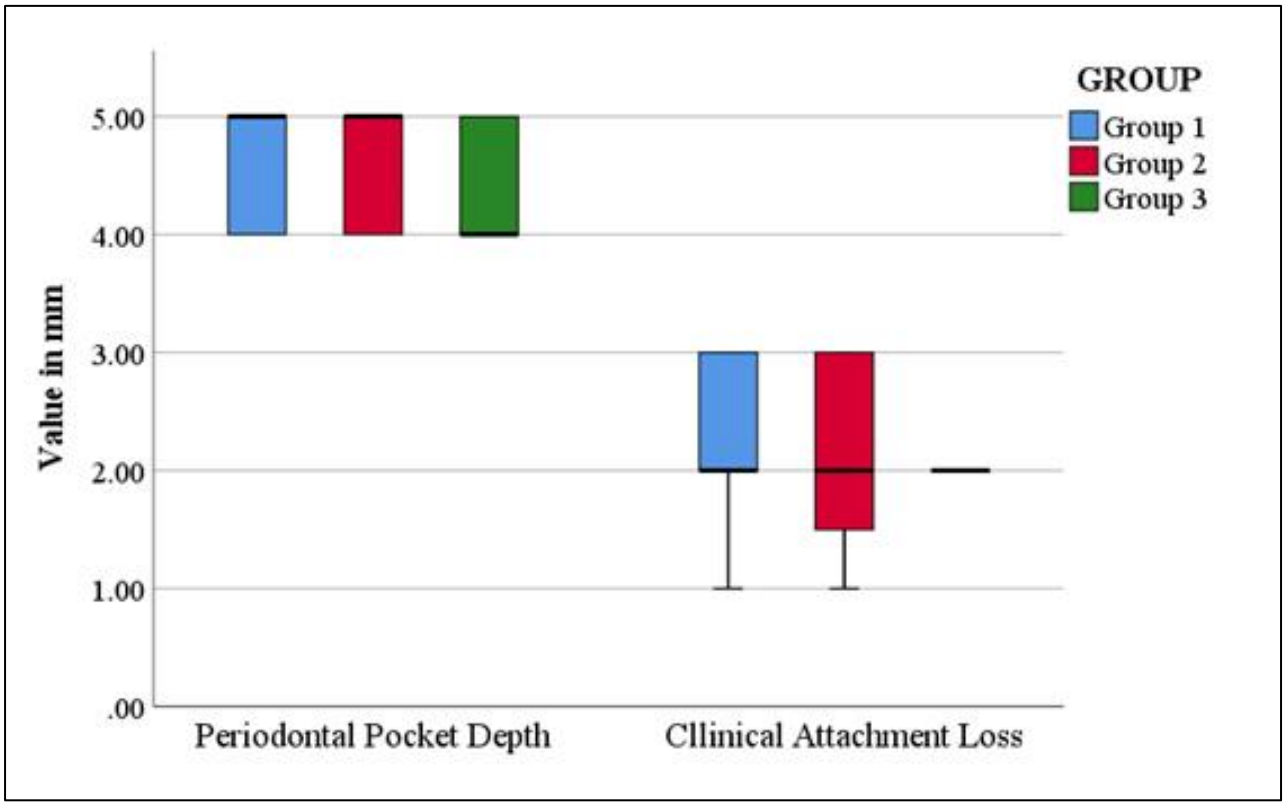

Table 3. Comparison of CFU.

	Group 1		Group 2		Group 3		Chi-square value	P-value[a]
	Median	IQR	Median	IQR	Median	IQR		
Colony forming units per plate	71.0	61.0-81.0	35.5	29.5-42.5	111.0	90.0-126.75	43.827	.001*

IQR- Inter-quartile range. [a]Kruskal-Wallis test. *p-value <.05 was considered statistically significant.

Figure 3. Colony forming units among 3 groups.

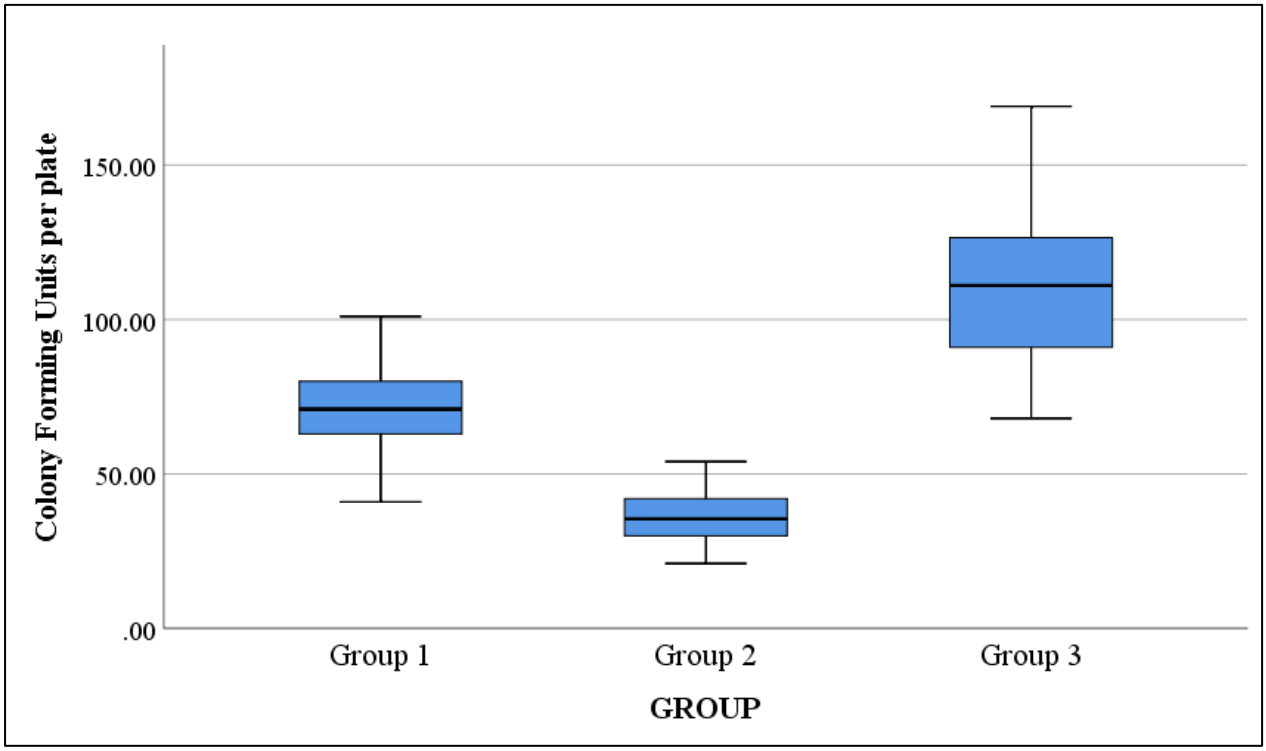

Table 4. Post hoc analysis (CFU).

Pair-wise	p-value
Group 1 vs Group 2	.001*
Group 1 vs Group 3	.001*
Group 2 vs Group 3	.001*

*p-value <.05 was considered statistically significant.

Figure 4: CFUs on Blue light agar plate

Figure 5: CFUs on 0.2 Chlorhexidine agar plate

Figure 6: CFUs on Hydrogen peroxide with Blue light agar plates

Discussion

Periodontitis is a multifactorial biofilm induced inflammatory disease and mechanical removal of the biofilm plays a crucial and essential part of the periodontal therapy. Oral bacteria from the biofilm can be released when dynamic dental instruments like water-air sprays, ultrasonic scaling instrument and high-speed rotary devices are used.[9]The bioaerosols posing a serious risk for contamination of the dental operatory and also as a source of transfer of various communicable diseases to the patients as well as the dental personnel and the treating dentist.[10]Though various protective barrier methods like gloves, masks, eyewear and scrubs are used as a means of protection from these bioaerosols. There is always a chance of cross contamination as studies have shown that bioaerosols remain suspended in the air for up to 4 hours.[3]

Pre-procedural rinses provides a viable and economical means of protection by reducing the bulk of the bacterial burden.[4] Various antimicrobial agents like Chlorhexidine, Povidone Iodine, herbal extracts are used as pre-procedural rinses. Chlorhexidine has always been considered as gold standard among pre-procedural rinses but comes with added disadvantage like staining, hypersensitivity etc. Though herbal mouth rinses have been used as an alternative to Chlorhexidine, results have not been consistent with that to Chlorhexidine. Thus the present study was planned to compare the effect of 0.2% Chlorhexidine, 3% Hydrogen Peroxide with blue light and blue light alone as agents to reduce aerolized bacteria.

The results of the present study demonstrates that the bioaerosols from the subjects who rinsed with 3% Hydrogen peroxide mouth wash followed by blue light irradiation (group 2) before ultrasonic scaling procedure harbored a significantly lower bacterial content than those subjects who used 0.2% Chlorhexidine mouth wash (group 1) or blue light alone (group 3).

In our study we have used only one blood agar plate on the patient's chest as compared to studies by Nayak et al[5], Gupta et al[11], Ammu et al[12], Rekha Rani et al[13] who used blood agar plates on patient's and operator's chest. Studies have revealed that pre-procedural rinses with 0.2% Chlorhexidine had a substantial reduction in the production of bioaerosols as demonstrated in the reduction of CFUs in blood agar plates. In the present study subjects (group 1) who rinsed with 0.2% Chlorhexidine showed a substantial reduction in CFUs

(Median CFUs 71, IQR- Inter-quartile range 61.0-81.0, p<.05) and the result obtained in our study are consistent with studies by Gupta et al[11], Ammu et al[12], Nayak et al[5], Rekha Rani et al[13]This may be attributed to the antimicrobial activity of Chlorhexidine wherein it increases the permeability of bacterial cell wall leading to precipitation of protein and nucleic acid.

Group 2 subjects (H_2O_2 and blue light) demonstrated a far more superior reduction of CFUs when compared to the other groups. (Median CFUs 35.5, IQR- Inter-quartile range 29.5-42.5 ,p<.05) To the best of our knowledge there are no clinical studies on the use of 3% H_2O_2 with blue light as a pre-procedural rinse to reduce aerolized bacteria, the results will be compared to the in vitro studies. Mahdi et al[14] showed enhanced bactericidal effect when 3% H_2O_2 was used along with blue light. Kunz et al[15] demonstrated that when 3% H_2O_2 was used with blue light as an adjunct to riboflavin had a potent antimicrobial activity killing all the bacteria within the biofilm. The results of our study are in accordance with the results of the above mentioned studies. The mechanism of the antimicrobial activity of the combination of 3% H_2O_2 with blue light can be explained that the bacterial toxicity of H_2O_2 is caused by the production of a hydroxyl radical during the oxidation of divalent ions. The oxidation of proteins and lipids damages the bacterial cell membrane. More so, it is an uncharged, covalent molecule that rapidly combines with water to facilitate the passage of hydroxyl radicals into the biofilm's deepest layer.[16]

Group 3 subjects (blue light alone) also demonstrated a significant reduction in the CFUs in our study. (Median CFUs 111.0, IQR- Inter-quartile range 90.0-126.75, p<.05) As there are no clinical studies the results of our study will be compared to the in vitro studies. Blue light (400-500nm wavelength) exerts a wide bacteriocidal effect on various periodonto pathogenic micorganisms. Feuerstein et al[17] showed that broad band blue light exerted a phototoxic effect on P. gingivalis and F. nucleatum. Chui et al[18] reported that blue light led (452-470nm) was able to inhibit the growth of P. gingivalis suspension. The presence of large concentrations of endogenous photosensitizers such cytochromes, porphyrins, flavins, and NADH within bacterial cells is the mechanism underlying the bactericidal impact of blue light.

Within the constraints of this study, it can be concluded that 3% H_2O_2 with blue light is a far more superior and effective pre-procedural rinse than 0.2% Chlorhexidine or blue light in reducing aerosol cross contamination during the use of ultrasonic scaling. Further longitudinal studies are warranted with larger sample size to substantiate the results obtained from this study.

Conclusion

The formation of bioaerosols was shown to be significantly reduced by 3% H2 O2 and LED Blue light as compared to the commonly used antibacterial 0.2 Chlorhexidine or Blue light. Therefore the pre-procedural rinses should be incorporated as a mandatory clinical practice in reducing bioaerosols and providing a safe dental experience.

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