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A Comparative Qualitative Analysis of Ozonised Water and Povidine Iodine as a Pre-Procedural Rinse in Chronic Periodontitis – A Randomized Controlled Clinical Trial.

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ABSTRACT

AIM: Dental procedures create aerosols that are made of air, water, patient's saliva and also bacteria, fungi and viruses. Aerosols may spread infectious diseases. Limiting the production of contaminated aerosols could help to prevent disease transmission in a dental setting. Hence, the aim of this trial was to compare the efficacy of mouth rinses 0.2% Povidone iodine (PVP), ozonized water (4ppm) and saline in reducing the bacterial load in aerosol samples collected during ultrasonic dental scaling.

METHODOLOGY: 30 patients were assigned to three groups through computer generated randomization, and were subjected to scaling after rinsing with either 4ppm ozone water, 0.2% PVP and saline to compare and evaluate their efficacy. Fresh blood agar plates were used for air sampling, which were sent for culturing and microbiological examination.

RESULTS: The results demonstrated high percentage reduction of aerobic colony forming units (CFUs) in all three groups. In aerobic culturing, OZ showed the highest reduction in all three positions.

CONCLUSION: The importance of aerosol and splatter mitigation strategies for all dental procedures, including those associated with dental ultrasonic use is increased with present pandemic situation. Ozone therapy is quite predictable and conservative.

KEY WORDS: Aerosols, cross infection; infection control, culturing, pre-procedural rinse, ultrasonic scaling, ozone therapy.

I. INTRODUCTION

Dental procedures are known to produce aerosols (droplets, droplet nuclei, and splatter). The contamination of these aerosols with pathogenic microorganisms are an important consideration for infection control and occupational health hazard to patients or staff in the confines of the dental clinic. Infective agents may include bacteria, viruses, fungal organisms and possibly even prions.1 The COVID 19 pandemic has resulted in difficult situations for infection control in the dental office thus posing a risk of exposure to both dentist as well as patients.

Aerosols are solid and liquid particles with particle size 50 μm or less and suspended in air by machines, instruments or humans.2 Splatter is usually described as a mixture of air, water, and/or solid substances, such as carious tissues, dental fillings fragments, sandblasting powder, etc.3 The size of water droplets in splatter range from 50 μm to several millimetres and can be easily seen by naked eye.4 Because of their bigger size, they remain air borne very briefly and hence rarely enter the respiratory passages.5 As documented in studies, the greatest infection causing potential is carried by aerosols as they can stay air borne for longer time and can easily enter the respiratory passages.6 Bioaerosol compositions are heterogeneous; they contain blood, microorganisms, mucosal cells, restorative materials, tooth particles and large quantities of saliva.7 The mean level of bioaerosols depends on

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the procedures; higher levels were observed for cavity preparation (24–105 CFU/m3) and for ultrasonic scaling (42–71 CFU/m3), and lower levels were reported for extraction (9–66 CFU/m3) and for oral examination (24–62 CFU/m3).8 Studies reported longer duration of presence of aerosols in clinical environment with long time survival of bacteria and viruses in these aerosols for as long as six days.3

There is a compelling need for greater attention towards tested methods to eliminate/ reduce the risk of aerosol contamination from aerosol generating procedures such as scaling , polishing, root debridement and restorative / prosthodontic preparations. Different materials and procedures are recommended for reducing bio aerosol contamination by the Centre of Disease Control and Prevention (CDC) and American Dental association (ADA) such as use of personal protective equipment, dental staff immunization, surface decontamination, equipment sterilization and dental unit water line treatment.1 A range of approaches can be used to reduce production of potentially infectious aerosols during dental procedures such as using anti-microbial mouthwash, placing a rubber sheet dam, isolate the treatment zone from saliva using a saliva ejector, high- volume evacuator , general ventilation and decontamination of air-borne aerosols by ultraviolet light. A variety of oral antiseptic rinses have been suggested in recent literature for pre-procedural use to reduce viral transmission. Chlorhexidine gluconate, ethanol, essential oils, povidone-iodine (PVP-I), hydrogen peroxide (H2O2) chlorinated water, hypertonic saline, bioflavonoids, cyclodextrins and cetylpyridinium chloride have been tried.9

Antimicrobial mouthwashes used prior to aerosol generating procedures can reduce the production of infectious aerosols. They reduce the pathogenic concentration of saliva there by reducing the microbial counts in the aerosols during Aerosol Generation Procedures (AGP‘s). PVP-I oral rinses demonstrated complete inactivation of SARS-CoV-2 at concentrations between 0.5% and 1.5% and contact times as little as 15 seconds.10 Though PVP-I solutions at concentrations below 2.5% have been demonstrated to be safe for routine, repeated use in the oral cavity, they are not recommended for patients with active thyroid disease, pregnancy, anaphylactic allergy, and in patients undergoing radioactive iodine therapy.9

Ozone executes antimicrobial effect by destroying the cytoplasmic membrane due to ozonolysis of dual bonds and alteration of intracellular contents selectively for microbial cells sparing human body cells. Medical grade Ozone gas has a high oxidation potential as an antimicrobial agent against bacteria, viruses, fungi, and protozoa. Ozonated water (4 mg/l) for 10 sec was found effective for killing gram-positive and gram-negative oral microorganisms and oral Candida albicans in pure culture as well as bacteria in plaque biofilm and therefore might be useful as a mouth rinse to control oral infectious microorganisms in dental plaque.11 A single irrigation of Ozonized water was quite effective to inactivate microorganisms (Kshitish and Laxman).12 Chlorhexidine and ozone showed similar efficacy in reducing aerobic and anaerobic CFU’s in aerosols .It would be helpful to identify substitutes for PVP-I considering its restricted usage in certain population as mentioned above .

The purpose of this study was to compare the efficacy of mouth rises (4ppm ozonized water, 0.2 % PVP and normal saline in reducing bacteria in the dental aerosol at clinically recommended convenient time scales.

II. MATERIALS AND METHODS

The present study was a prospective single center comparative controlled randomized double blind clinical trial for the analysis of ozonized water and povidine iodine as a pre procedural rinse to reduce bacterial load in dental aerosols during scaling and root planing in chronic periodontitis patients. The current trial followed the ethical guidelines of the Institutional Research and Ethical Committee, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru, India. Participants satisfying the inclusion criteria were informed about the purpose of the study, nature of the procedure and possible discomforts and risks and obtained an informed consent.

SOURCE OF DATA

Patients referred to the outpatient department of periodontology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore and satisfying the inclusion and exclusion criteria were selected for the study.

SUBJECT SELECTION

30 patients who are systemically healthy, satisfying the inclusion criteria were selected Inclusion criteria**: p**atients > 18 years of age; healthy or treated and controlled periodontal conditions, systemically healthy, not under medications, minimum of 20 permanent functional teeth and plaque index score and gingival index score between 1-3. Patients with following criteria

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were excluded: pregnant and lactating women, immunocompromised subjects, patients taking drugs or need prophylactic antibiotics, history of oral prophylaxis or mouthwash used within the past 3 months, consumption of tobacco in any form, smokers and alcoholics.

SAMPLE SIZE CALCULATION

The sample size was calculated using G power software 3.1.9.4 with effect size of 0.5 and α error of 0.05 and power (1- β) of 0.80 that gave a total sample size of 30 subjects. Through computer generated randomization 30 patients were randomly assigned into three groups; Group A: 10 patients rinsing with normal saline water for 1min; Group B: 10 patients using 5ml of 0.2 % PVP – I for 1min and Group C: 10 patients using 4mg/l ozonised water for 1min. In order to maintain blinding the randomization and allocation to groups was concealed until the analysis of results was computed.

Patients were analysed in the same standard operatory which followed the approved fumigation protocol between each patient. The operatory was a closed room with a single dental unit. Fresh blood agar plates were used for air sampling, Blood agar plates were used to collect the gravitometric settling of aerosols which were then transported to Department of Microbiology for culturing and microbiological laboratory examination. For the aerosol capture, three standard reference locations were selected from the patient's mouth: the left side from the mouth at a distance of one feet, the right side from the mouth at a distance of one feet, and the area in front of the patient's foot at a distance of two feet. Patients were instructed to rinse for 1 minute with one of the pre-procedure rinses that were assigned to them. Supragingival scaling was performed on all patients in the maxillary segment (tooth number 13 to 23) for 15 min by the operator. During the scaling process, a saliva ejector was employed, and the coded blood agar plates were left in place at the predetermined spots for 30 minutes. To keep the room clean and avoid cross infection, the trial was limited to just one patient per day. A masked operator performed the identical operation on all individuals. Blood agar plates (BHI) were collected and incubated aerobically at 37°C for 18-24h. Colonies were counted using the colony counter device by the masked examiner.

III. STATISTICAL ANALYSIS

All statistical procedures were performed using Statistical Package for Social Sciences (SPSS) 20.0. Calculations for power (80%) of study were performed before the commencement of the study. All quantitative variables expressed in mean and standard Deviation. Qualitative variables will be expressed in percentages. Shapiro-Wilk test was used for testing the normality assumption of the quantitative data. One Way ANOVA test was used for association between variables followed by tukeys post hoc comparison. Probability value ($p < 0.05$) was considered statistically significant.

IV. RESULTS

In the current study, 30 patients were selected and were randomly allocated into Group A (NS), Group B (PVP-I), Group C (OZ) groups and each group consisted of 10 patients. At baseline all the 3 groups showed similar plaque index (1.0- 1.9) and bleeding index (10-20 %).

Intragroup comparison of ozone group,PVP-I group and NS group showed statistically highly significant CFU on the left side. (Table 1) At the right side, intergroup comparison of CFU between 3 groups, showed no statistically significant difference. At the left side intergroup comparison of CFU between 3 groups, showed statistically significant increased counts in the control group . At front side intergroup comparison of CFU between 3 groups, showed no statistically significant difference (Table 2). The Post hoc multiple comparisons between ozone group and PI group/ control groupshowed a statistically significantly decreased CFU in ozone group. Comparison between PI and ozone group showed a statistically significantly decreased CFU in ozone group. Similarly Comparison between ozone group and control group showed a statistically significantly decreased CFU in ozone group (Figure 1 , 2 and 3; Table 3)

V. DISCUSSION

The American Dental Association has recommended that contaminated aerosols or splatter should be controlled during dental procedures. COVID-19 has raised concerns regarding aerosols generated during dental procedures, including the length of time these aerosols remain airborne and the distance they can travel. The minimum droplet size necessary to transport SARS-CoV- 2 is unknown, but coronaviruses range from 70 to 120nm in size was studied by Cascella M et al 2020 and Kim et al 2020. A recent study of Wuhan Province hospitals during the COVID outbreak showed that out of five size ranges, the highest concentration of SARS-CoV- 2 particles was 0.25–1.0μm (submicron) and 2.5μm+ (fine or supermicron).13

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The results of both simulated and real-time tests confirm regions proximate to the source have the highest concentration of surface contamination and airborne particle concentrations. This is consistent with other studies showing the greatest contamination within 0.3 m (1 ft.) of the operative site, and that contamination/particle load decreases with increasing distance. Veena et al. 2015 found that contamination decreased by 50% at distances over 0.6m (2 ft.), and Bentley et al.10 found uniform bacterial contamination 0.6m (2 ft.) from the source. Collectively, these conclusions indicate that most particles generated during ultrasonic scaling are found within 0.6m (2 ft.) of the patient's mouth.14 While multiple prior studies show microbe-bearing splatter and aerosols generated during dental treatment often land in proximate regions (eg; patient's chest or provider PPE), recent research shows aerosols generated by ultrasonic scalers can travel up to 3.96m (13 ft.).15 Altogether, droplet and particulate contamination leading to viral transmission indicate that multiple barriers are necessary to reduce the risk of viral spread during dental procedures. Most of the studies showed that marked reduction in detectable droplets with high-volume evacuation (HVE) used during ultrasonic scaling for all droplet size ranges, indicating this is a critical tool for removing potentially virus-laden particles at the source.16,17 Evacuation devices are, therefore, necessary to protect the health of dental healthcare providers (DHCPs). Other splatter mitigation strategies include personal protective equipment to limit the risk of inhalation and exposure, and protective barriers to limit potential transmission via contact with contaminated surfaces. Preprocedural mouth rinses, such as chlorhexidine and cetylpyridinium chloride, can reduce bacterial load in dental aerosols by 68.4%.18

Ozone therapy is one of the modern non-medication methods of treatment. Ozone is an unstable gas and it quickly gives up nascent Oxygen molecule to form Oxygen gas. Due to the property of releasing nascent Oxygen, it has been used in human medicine since long back to kill bacteria, fungi, to inactivate viruses and to control hemorrhages.19 The role of microorganisms and host response in the etiology of periodontal disease is well established. Ozonated water (4 mg/l) was found effective for killing gram-positive and gram-negative oral microorganisms and oral Candida albicans in pure culture as well as bacteria in plaque biofilm and therefore might be useful as a mouth rinse to control oral infectious microorganisms in dental plaque. Thanomsub et al. 2002 tested the effects of ozone treatment on cell growth and ultra-structural changes in bacteria (Escherichia coli, Salmonella sp., Staphylococcus aureus and Bacillus subtilis). Nagayoshi et al. 2004 tested the efficacy of ozonated water on survival and permeability of oral micro-organisms. Gram negative bacteria, such as Porphyromonas endodontalis d Porphyromonas gingivalis substantially more sensitive to ozonated water than gram positive oral streptococci and c. albicans in pure culture. The previous studies that have evaluated the effects of different antiseptics in reducing aerosol contamination, have recommended CHX as the gold standard.20 Ozonated water has a half-life of about only20 min and will degrade back into oxygen very quickly, so it should be used within the first 5–10 min to assure its potency.11 Although the test groups significantly reduced the growth of CFUs compared to the control group. Ozone and povidine iodine preprocedural rinse showed a similarity in reducing aerosolized bacteria.

VI. LIMITATIONS

The limitations of this study should be considered in the interpretations of the results. The CFUs counted here are values that represent the bacteria capable of growth on blood agar plates. No attempt has been made to identify the type of bacteria, pathogenic or nonpathogenic/ aerobic or anaerobic. Moreover, viruses, fungi and specific bacteria require specialized media, which were not cultured in this study. One limitation of the present study is that it presents results for a single dental procedure, prophylaxis with an ultrasonic scaler that has been shown to have a great potential for aerosol generation in the dental office. However, other dental procedures can also generate a large amount of aerosol with infectious components launched into the dental environment, such as the air turbine hand piece, air-water from a three-way syringe and sodium bicarbonate jet.

VII. CONCLUSION

The importance of aerosol and splatter mitigation strategies for all dental procedures, including those associated with dental ultrasonic use is increased with present pandemic situation. Ozone therapy is quite predictable and conservative. Ozone therapy is beneficial. Treating patients with ozone therapy lessens the treatment time with an immense deal of variation and it eradicates the bacterial count more specifically. The treatment is completely painless and increases the patients' acceptability and compliance with minimal adverse effects.

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TABLES

Intra group comparison (OZONE GROUP)					
	Mean	**SD**	**95% CI interval**		**P value**
			Lower bound	**Upper bound**	
Right	31.40a	11.15	23.41	39.38	<0.001**
Left	64.90ab	25.60	46.58	83.21	
Front	34.00b	12.4	25.05	42.94	
Intra group comparison (0.2% POVIDINE IODINE GROUP)					
Right	39.20a	23.99	22.03	56.36	0.001**
Left	104.60ab	46.52	71.31	137.88	
Front	57.10b	35.46	31.73	82.46	
Intra group comparison (CONTROL GROUP)					
Right	48.40a	21.45	33.05	63.74	<0.001**
Left	133.20ab	31.01	111.02	155.37	
Front	45.10b	23.02	28.62	61.57	

Table 1: Intra group comparison (OZONE GROUP) ,0.2% POVIDINE IODINE GROUP and CONTROL GROUP),* Significant, **Highly significant; same alphabets indicate significant difference across the group (LSD post hoc)

Comparison of CFU between three groups at right side					
Groups	**Mean**	**SD**	**95% CI interval**		**P value**
			Lower bound	**Upper bound**	
Ozone group	31.40	11.15	23.41	39.38	0.17
0.2% povidine iodine group	39.20	23.99	22.03	56.36	
Control group	48.40	21.45	33.05	63.74	
Comparison of CFU between three groups at left side					
Ozone group	64.90	25.60	46.58	83.21	0.001*
0.2% povidine iodine group	104.60	46.52	71.31	137.88	
Control group	133.20	31.00	111.02	155.37	
Comparison of CFU between three groups at front side					

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Ozone group	34.00	12.49	25.05	42.94	0.14
0.2% povidine iodine group	57.10	35.46	31.73	82.46	
Control group	45.10	23.02	28.62	61.57	

		Mean Difference	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Ozone group	**0.2% povidine iodine group**	-39.70*	15.87	**0.019**	-72.27	-7.12
	Control group	-68.30*	15.87	**0.000**	-100.87	-35.72
0.2% povidine iodine group	**Ozone group**	39.70*	15.87	**0.019**	7.12	72.27
	Control group	-28.60	15.87	0.083	-61.17	3.97
Control group	**Ozone group**	68.30*	15.87	**0.000**	35.72	100.87
	0.2% povidine iodine group	28.600	15.87	0.083	-3.97	61.17

Table 2: Comparison of CFU between three groups at right side , left side and front side ; # One wayANOVA
Table 3: Post hoc Multiple Comparisons, *. The mean difference is significant at the 0.05 level.* Significant, **Highly significant; same alphabets indicate significant difference across the group (LSD post hoc)

FIGURES

Figure 1: Agar plates showing colony forming units (CFU) in Povidone iodine group (A. Right side B. left side C. In front of the patient.)

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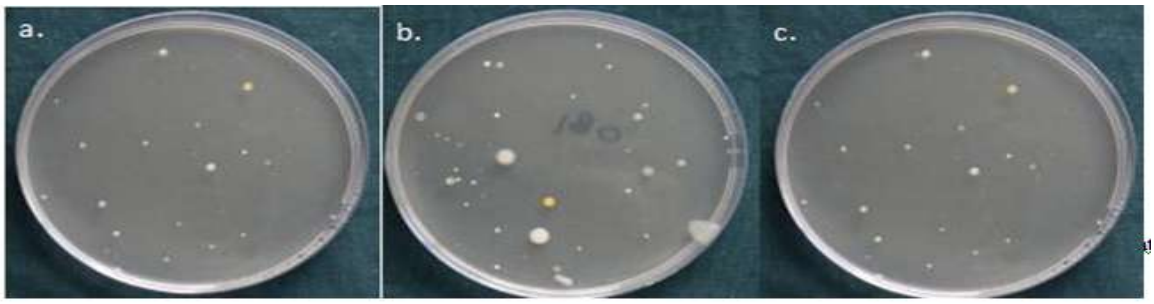

Figure 2: Agar plates showing colony forming units (CFU) in ozone group (A. Right side B. left side C. In front of the patient)

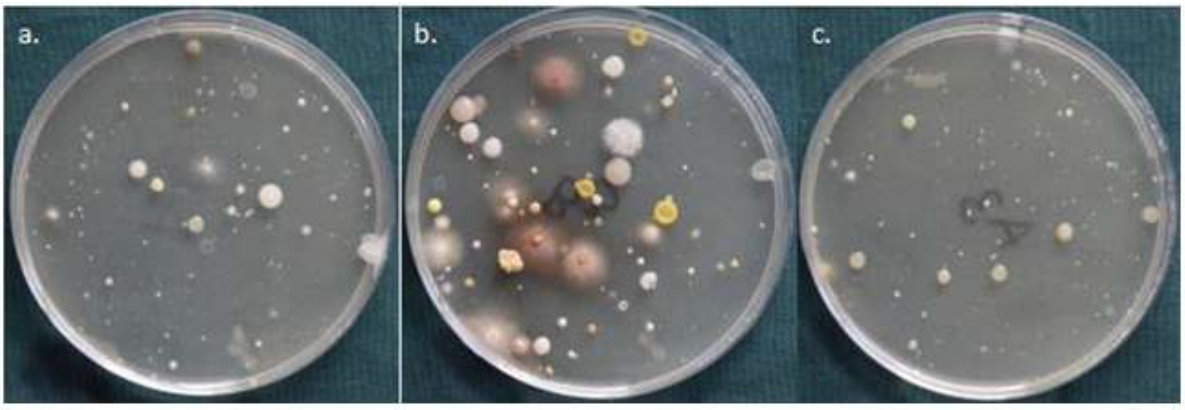

Figure 3: Agar plates showing colony forming units (CFU) in control group(A. Right side B. left side C. In front of the patient.)