



RESEARCH ARTICLE

COMPARATIVE EVALUATION OF POMEGRANATE JUICE AND CHLORHEXIDINE GLUCONATE AS ULTRASONIC LIQUID COOLANTS ON DENTAL AEROSOLS: A RANDOMIZED CONTROL CLINICAL AND MICROBIOLOGICAL TRIAL

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ABSTRACT

Aim: To evaluate the effect of Pomegranate juice and Chlorhexidinegluconate as ultrasonic liquid coolant on aerosols in comparison with normal saline clinically and microbiologically. **Method:** Thirty chronic periodontitis patients were divided into Group 1: normal saline, Group 2: pomegranate juice, Group 3: 0.12% chlorhexidinegluconate, used as ultrasonic liquid coolants. One blood agar plate was kept in fumigated chamber 10 minutes before scaling. Calibrated face shield was used by clinician to evaluate face contamination during scaling. Two agars at distance of 0.4 meters on either side and one 2 meters behind dental chair during scaling were evaluated for colony forming units (CFU) on agar. **Results:** At baseline, no significant CFUs were detected. Mean CFU reduction in Group 3 (26.53 ± 6.65) and group 2 (29.6 ± 3.03) but results obtained were not statistically significant. CFU were highest in group 1 (123.9 ± 9.13). More CFU were found on agar which were kept on right side in all the three groups. The area near the ala of the nose and inner canthus of eye has more contamination on face shield. **Conclusion:** Pomegranate juice is potent antimicrobial agent and can be used as an ultrasonic liquid coolant during scaling.

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INTRODUCTION

In the dental clinics, bioaerosols are generated during the use of hand pieces, ultrasonic scalers, air and water syringes, and possibly also through the use of lasers (Mckinley, 1994). Bentley *et al.* (1994) evaluated the distribution of spatter and aerosols generated by high speed instrumentations, showing that contamination from spatter and aerosol dissemination remains a significant hazard for dental personnel. Aerosols term was first used by Micik *et al.* in his pioneering work in aerology (Micik, 1969). Aerosols are the combination of both liquid and solid particles, majority of the particles are less than 100 microns and when the water gets evaporated, they form 'droplet nuclei' which is composed of saliva, dried serum and microorganisms. The size of the droplet nuclei varies from 0.5 to 10 microns which can reach pulmonary alveoli or float in the air for several hours. Sotiriou *et al.* suggested that dental drilling procedures aerosolize saliva and produce particles small enough to penetrate deep into the lungs (Sotiriou *et al.*, 2008; Zymańska, 2007). So aerosols are an important consideration for infection control and occupational health, since infectious agents could be transmitted via aerosols to dentist, patients or dental staff in the confines of the dental units.

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Infective agents include bacterias, viruses, fungi and possibly even prions. There is little evidence of transmission of such viruses via aerosols causing disease among dentists. Viruses, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), influenza virus and Herpes virus, could easily be contained amongst the smallest of aerosols. Similarly, prions, smaller infective particles causing diseases such as spongiform encephalopathy, could theoretically also be contained in such aerosols (Grenier, 1995). World health organization has reported 2.5% of human immunodeficiency virus (HIV) and 40% of Hepatitis B and Hepatitis C infections in healthcare staff by occupational contacts (Geneva, 2002). Dentist are under constant risk of contamination from the different bacterias and other infective aerosols and splatter which may be generated from tooth debris, plaque, calculus, dental materials, spray water, saliva and blood during dental procedures (Geneva, 2002). Checchi *et al.* (1991) showed that exposed areas of the dentist's face are at risk with contaminated particles. So different protective measures are used during dental procedures which includes gloves, masks, face shield, high vacuum suction, aerosol reduction devices, use of antimicrobial agents as pre procedural mouth rinses and ultrasonic liquid coolants during ultrasonic scaling. Chlorhexidine is commonly used as antimicrobial agent. Chlorhexidine is considered as a gold standard and used as pre

procedural mouth rinse in different studies (Snophia *et al.*, 2011). Jawade *et al.* used chlorhexidine as ultrasonic liquid coolant during scaling and evaluated aerosol reduction during scaling.⁹ Now a day the era is moving towards the use of herbal agents in dentistry. One of the herbal products which are now days used in dentistry is pomegranate. Pomegranate is a shrub which is found in Asia on a large scale. It has antibacterial, antifungal, antiviral and antioxidant properties. There are few studies in literature using pomegranate as antimicrobial agent.

Aim: To evaluate the effect of Pomegranate juice and Chlorhexidine gluconate as ultrasonic liquid coolant on aerosols in comparison with normal saline using clinical and microbiological analysis.

MATERIALS AND METHODS

Study design

The study was a randomized control double blind study conducted in Department of Periodontics, Yogita Dental College and Hospital, Khed.

Inclusion criteria

- Age- 20-50 years.
- Minimum of 20 permanent teeth.
- Patients with chronic periodontitis.

Exclusion criteria

- Subjects with systemic diseases.
- Pregnant and lactating mothers.
- Subjects taking any medication which alters the periodontal status from past six months.
- Subjects undergoing any periodontal therapy for past six months.
- Subjects who were allergic to chlorhexidine and pomegranate.

Study Protocol

The study was ethically approved by the Ethical Committee of Yogita Dental College and Hospital, Khed. A written consent was signed by all the participants before the commencement of the study. The patients who fulfilled the inclusion criteria were included in the study. Study included 30 Patients aged 20-50 years with chronic periodontitis and were randomly divided into 3 groups, Group 1- Normal Saline group, Group 2- Chlorhexidine Gluconate (CHX) group Group 3- Pomegranate Juice group (PGJ). 10 patients were divided randomly in each group using lottery method. These agents were used as ultrasonic liquid coolant in dilution with distilled water in the ratio of 1:1 during ultrasonic scaling.

Clinical Parameters

Probing Pocket Depth (PPD), Clinical Attachment Loss (CAL), Plaque Index (PI) by Sillness and Loe (1964), Gingival Index (GI) by Loe and Sillness (1963) were recorded using UNC 15 probe.

Ultrasonic Scaling Procedure

Before the start of the ultrasonic scaling a closed chamber was fumigated, after 24 hours one blood agar plate was kept in the

closed chamber for 10 minutes before ultrasonic scaling, to evaluate the non-contamination of the area before the start of scaling. (Fig 1) After 10 minutes the agar plate was removed. Two blood agar plates were kept at a distance of 0.4 meters away on either side of the patient and one agar plate was kept 2 meters behind the patient's head and patient's ultrasonic scaling was executed for 20 min by the clinician (Fig 2) During scaling the same rate of flow of each coolant was maintained. A calibrated face shield was used by the clinician during scaling to evaluate the area of contamination of the clinician's face during scaling. High vacuum suction was used during each scaling. To assure that the room was free from aerosols, only one patient was treated per day. After the treatment, three coded blood agar plates were left uncovered for 20 min at the pre-designated sites for gravitometric settling of airborne bacteria. After gravitometric settling of aerosols, blood agar plates were transferred to laboratory for incubation at 37°C for 48 hours.

Face shield Calibration

Fourteen squares with 1 cm surface area were determined on a checked A4 paper as a pattern for each side of the face, three squares were located around the eye (inner corner, outer corner and middle of the eye), one on the cheek, one lateral to the ala and two around the mouth (commissure and middle of lips). The squares were numbered 1 to 7 on right side and lettered A to G on left side of the face (Fig 3)

Face shield and blood agar plate contamination determination

The calibrated face shield borders were matched with the borders of the checked paper. Each shield was investigated separately, in a way that visible particles on it were counted, using a magnifier equipped with a small light ($\times 2$). Microbiological analysis- The blood agar plates were analyzed after incubation for CFU by colony counting procedure with the help of colony counter device by the microbiologist (Fig 4)

Statistical Analysis

The data was collected and tabulated in Microsoft excel format and the statistical analysis was done using SPSS 18 software (IBM, Armonk, NY, USA) and was expressed as mean \pm SD. The total sample size was 30 patients with chronic periodontitis. The ANOVA test was used for comparison of Normal Saline, Chlorhexidine Gluconate and Pomegranate Juice. Additionally Post-hoc Tukeys test was used. One way analysis of variance and Duncan tests was used for evaluation of contamination of clinician's face by aerosols on the face shield during scaling.

RESULTS

The mean CFU on all the sides of group 1 is depicted in (Table 1) group 2 in (Table 2) and group 3 in (Table 3). There was highest CFU reduction in group 3 followed by group 2 and least in group 1, the CFU values of group 2 and 3 were not statistically significant on comparison, which showed that pomegranate is equally efficient in reducing the CFU in aerosols as that of chlorhexidine, when used as ultrasonic liquid coolant during scaling. The CFU on right side of group 1, group 2 and group 3 are depicted in (Table 4) and there was statistical significant difference on comparison of group 1 with

group 2 and 3, but there was no statistical difference on comparison of group 2 and 3, also among all the sides the CFU were highest on the right side. The CFU on left side of group 1, group 2 and group 3 are depicted in (Table 5) and there was statistical significant difference on comparison of group 1 with group 2 and 3, but there was no statistical difference on comparison of group 2 and 3.

The CFU on behind side of group 1, group 2 and group 3 are depicted in (Table 6) and there was statistical significant difference on comparison of group 1 with group 2 and 3, but there was no statistical difference on comparison of group 2 and 3. (Fig 5).

Table 1. Colony Forming Units on all sides in Normal Saline group

Mean Colony Forming Units					
	N	Minimum	Maximum	Mean	Std. Deviation
Right	10	142	180	164.90	13.220
Left	10	118	139	126.90	8.006
Behind	10	71	88	80.00	6.200

Group 1= Normal Saline

Table 2. Colony Forming Units on all sides in Pomegranate Juice group

Mean Colony Forming Units					
	N	Minimum	Maximum	Mean	Std. Deviation
Right	10	38	50	44.40	4.142
Left	10	25	35	30.10	3.071
Behind	10	11	17	14.30	1.889

Group 2 = Pomegranate Juice

Table 3. Colony Forming Units on all sides in Chlorhexidine Gluconate group

Mean Colony Forming Units					
	N	Minimum	Maximum	Mean	Std. Deviation
Right	10	36	45	40.60	3.062
Left	10	24	30	27.00	2.261
Behind	10	10	14	12.00	1.333

Group 3 = Chlorhexidine Gluconate

Table 4. Comparison between Colony Forming Units of Group 1, Group 2 and Group 3 on Right Sides.

Intergroup Comparison on Right Side						
ANOVA followed by post hoc test Tukeys						
(I) groups	(J) groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
group1	group2	124.300*	3.663	.000	115.22	133.38
	group3	120.500*	3.663	.000	111.42	129.58
group2	group1	-124.300*	3.663	.000	-133.38	-115.22
	group3	-3.800	3.663	.560	-12.88	5.28
group3	group1	-120.500*	3.663	.000	-129.58	-111.42
	group2	3.800	3.663	.560	-5.28	12.88

*. The mean difference is significant at the 0.05 level.

Table 5. Comparison between Colony Forming Units of Group 1, Group 2 and Group 3 on Left Sides

Intergroup Comparison on Left Side						
ANOVA followed by post hoc test Tukeys						
(I) groups	(J) groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
group1	group2	99.900*	2.290	.000	94.22	105.58
	group3	96.800*	2.290	.000	91.12	102.48
group2	group1	-99.900*	2.290	.000	-105.58	-94.22
	group3	-3.100	2.290	.379	-8.78	2.58
group3	group1	-96.800*	2.290	.000	-102.48	-91.12
	group2	3.100	2.290	.379	-2.58	8.78

*. The mean difference is significant at the 0.05 level.

Table 6. Comparison between Colony Forming Units of Group 1, Group 2 and Group 3 on Behind Sides

Intergroup Comparison on Behind Side						
ANOVA followed by post hoc test Tukeys						
(I) groups	(J) groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
group1	group2	68.000*	1.709	.000	63.76	72.24
	group3	65.700*	1.709	.000	61.46	69.94
group2	group1	-68.000*	1.709	.000	-72.24	-63.76
	group3	-2.300	1.709	.383	-6.54	1.94
group3	group1	-65.700*	1.709	.000	-69.94	-61.46
	group2	2.300	1.709	.383	-1.94	6.54

*. The mean difference is significant at the 0.05 level.

The mean areas of contamination on the face shield is evaluated on the right and left side of the face. The areas lateral to the ala of the nose and inner corner of eye were more contaminated than the other areas, Zygoma was the least contaminated area. There was no significant difference between right and left side of the face (Fig 6).

DISCUSSION

Periodontitis is a multifactorial disease, which contains different microorganisms leading to destruction of surrounding tissues. Dental plaque contains different species of microorganisms, which are responsible for periodontal diseases.



Fig. 1. Blood agar position before ultrasonic Scaling

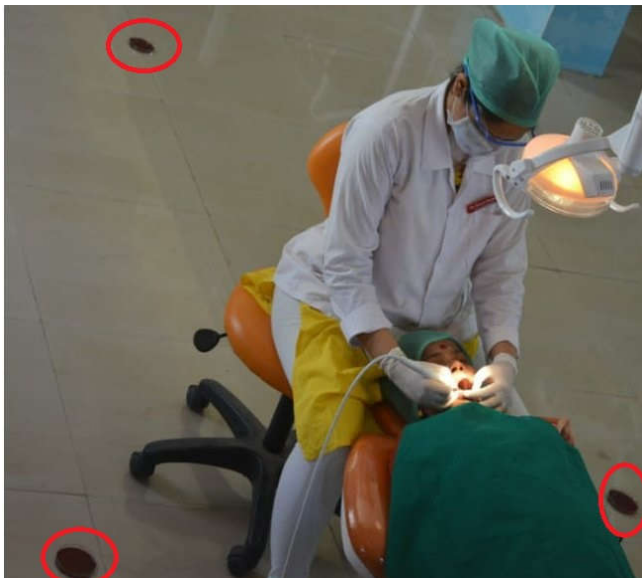


Fig 2. Blood agar position during ultrasonic scaling

Complete mechanical debridement is necessary for maintaining the healthy periodontium, which can be achieved by doing scaling and root planing. In dental practice, various dental procedures like ultrasonic scaling, crown preparation, caries excavation etc, cause production of aerosols, which contains potentially infectious blood borne and airborne pathogens (Swaminathan, 2013).

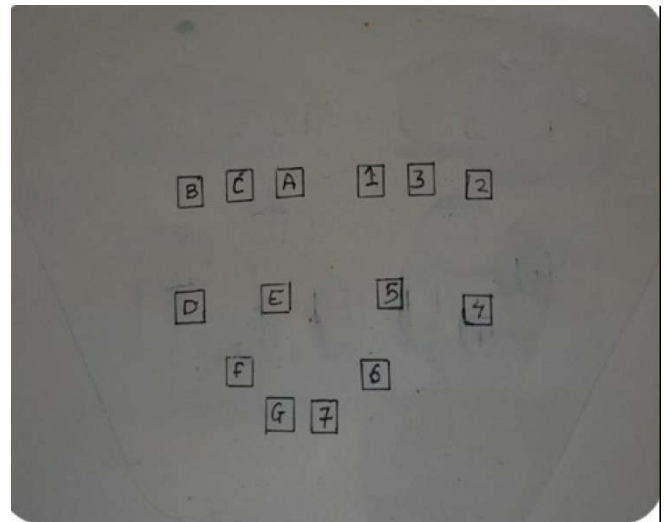


Fig 3. Evaluation of the aerosols on calibrated face shield using a template made on A4 size calibrated page

	Right	Left	Behind
Normal Saline			
Chlorhexidine			
Pomegranate			

Fig 4. Colony forming units after incubation

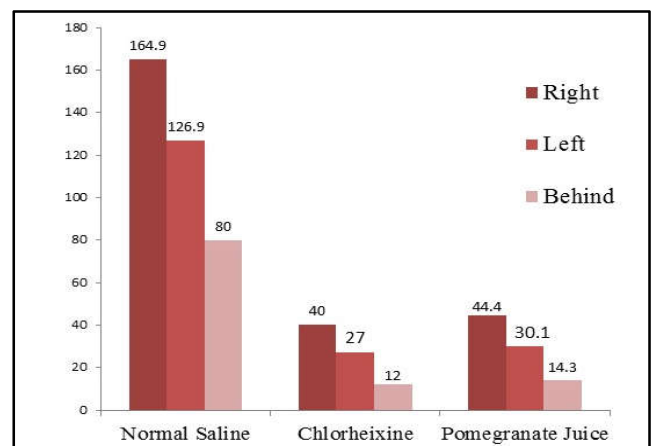


Fig. 5. Comparison of colony forming units formed on blood agar plates in three groups after ultrasonic scaling

It is well proven and accepted fact that pre-procedural rinse with chlorhexidine will reduce the bacterial count but the depth of pocket penetration is less than 2 mm (Braun, 1992; Pitcher, 1980; Wunderlich et al., 1984). The average length of universal ultrasonic scaler tip is 7mm (Nosal et al., 1991). The water coolant of the ultrasonic unit does extend apically as far as the probe tip thereby providing coolant at the tip of the instrument. Jawade et al. in a randomized study concluded that use of chlorhexidine as an ultrasonic coolant significantly reduces the microbial counts in aerosols produced due to ultrasonic scaler (Jawade et al., 2016).

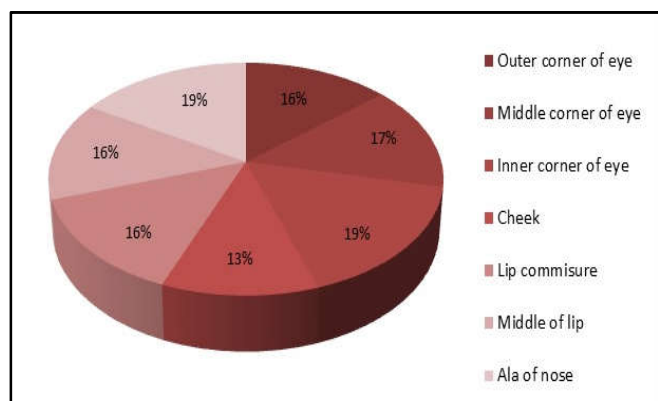


Fig 6. Comparison of different areas of contamination on face shield

The long term inhalation of pomegranate juice and chlorhexidine gluconate will not affect the operator's health because as studies have proven that the aerosols linger only till 30 min (Larato *et al.*, 1967), CHX and PGJ are used in solution form and in low concentration in study. The mean Colony Forming Units (CFU) were highest in the Normal saline group and almost equal in the Chlorhexidine and Pomegranate groups. The most abundant polyphenols in pomegranate juice are hydrolysable tannins called Punicalagins. The CFU were maximum on right side, as the operator included in this study was right handed and the patients were asked to tilt his/ her face towards operator's side. This result is similar to the result shown in the study by Jawade *et al.* (Hinds, 1982; Larato, 1967; Legnani *et al.*, 1994; Miller *et al.*, 1971; Miller, 1978).

On the face shield, the areas lateral to the ala of the nose and the inner corner of eye were more contaminated than the other areas. Since the dentist gets nearer to the patient during treatment to have a better view, it is not surprising to have more contamination in central areas of the face. Aerosol droplets are 50 μm or less in diameter and move 15-120 cm from patient's oral cavity resulting in more contamination of areas lateral to ala of the nose and inner corner of eye as compared to the other areas of the operator's face (Bennett, 2000; Leggat, 2001). The result of this study is similar to the results of a study by Nejatidanesh *et al.*¹⁷ Face shield is most effective and cheapest device to prevent aerosol contamination.

Conclusion

The ultrasonic scaling produces aerosols and can lead to different air borne diseases and it can be prevented by using different antimicrobial agents as ultrasonic coolants during ultrasonic scaling. Pomegranate juice is a potent antimicrobial agent and is equally effective in reducing the aerosols when compared with chlorhexidine. Hence pomegranate juice can also be used as an ultrasonic liquid coolant during ultrasonic scaling. Different protective devices can be useful to prevent contamination of the dentist, patients and the dental staff and it is an easy way to prevent contamination caused by microorganisms.

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