Efficacy of Two Mouth Rinses in Reducing Aerosol Bacterial Load during Ultrasonic Scaling

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Authors’ contributions

This work was carried out in collaboration among all authors. Author AA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MH and SAB managed the analysis of the study. Author BF performed the statistical analysis. Authors Fakhuruddin and FA managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aims: The aim of this study was to compare the efficacy of two mouth washes namely Chlorhexidine 0.2% and 5% green tea mouth rinse when used as pre-procedural rinses in reducing the number of CFU in aerosol generated during ultrasonic scaling.

Study Design: Quasi experimental study.
Place and Duration of Study: Department of Periodontology, Ziauddin college of Dentistry, Ziauddin University, Karachi, between January 2019 to August 2019.
Methodology: 70 subjects were recruited in this study (43 males, 27 females: age range 18-65 years with presence of minimum 20 permanent functional teeth, less than 5 mm mean probing depth and plaque and gingival score between 1-3. Subjects were randomly divided into two groups, group 1 and group 2. Each group comprised of 35 patients. A split mouth design technique was used for collecting the aerosol samples on blood agar.
plates. For every patient, there was a control side and test side of mouth. Ultrasonic scaling was done on control side without pre procedural rinsing and vice versa. Group 1 used 0.2% Chlorhexidine while group 2 rinsed with 5% green tea mouthwash. The blood agar plates were then transported to Microbiology lab and incubated for counting the colony forming units (CFU).

**Results:** Significant reduction of CFU occurred with pre procedural rinsing with both mouthwashes as compared to non-rinsing before ultrasonic scaling and 0.2% Chlorhexidine found to be superior to 5% green tea in reducing bacterial load in aerosol samples.

**Conclusion:** Pre procedural mouth rinsing with effective mouthwashes significantly reduces aerosol contamination and should be used before all dental procedures that results in formation of aerosols and splatters along with some other infection control protocol to minimize the risks of cross infection in clinical settings.

**Keywords:** Preprocedural rinsing; chlorhexidine; cross infection; ultrasonic scaling; aerosols; splatters; dental operatory; mouthwash; efficacy.

### 1. INTRODUCTION

The field of dentistry has many occupational hazards not only for dental professionals but for the patients as well [1]. These hazards are of different types including biological, physical, chemical, mechanical, psychological etc. [2]. Cross infection is one of the most important biological hazard found in dentistry and defined as the transmission of infectious agents between patients and staff within a clinical environment [3].

Airborne microorganisms and the transmission of body fluid are the two important biological risk factors found in clinical settings and associated with cross infection [4]. In clinical settings, cross infection and its control is the most important issue for health care providers [3]. In Dentistry, two important risk factors which increase the chances of cross infection are the surgical nature of dental practice and close contact with patients [5].

Infections known to affect dental practitioners are by various microorganisms including the Streptococci and Staphylococci groups, Mycobacterium Tuberculosis, Hepatitis B, Hepatitis C, HSV type 1, HIV, Mumps, Influenza and Rubella [6].

Frequent use of sharp instruments, close contact with the patients’ oral cavity and working with high-speed rotary instruments producing contaminated aerosols are some of the factors which make dental practitioners more exposed to biological hazards [7].

Air borne route has been documented in studies as one of the greatest potential source of spreading infection in medical and dental clinical settings [8]. Micik and colleagues used the terms “aerosol” and “splatter” as a result of their pioneering work on aerobiology [8].

Aerosols are defined as airborne particles less than 50 micrometers in diameter. Aerosol is created when high-powered devices need compressed air and water to work effectively [9]. Aerosol’s smaller size enables them to stay airborne for longer time period and carry the greatest infectious potential [10]. Splatter are defined as airborne particles larger than 50 μm in diameter. Because of their bigger size, they air borne very briefly [8]. Infectious pathogens including viruses, bacteria, fungi, and possibly even prions are reported to be present in aerosols [11]. Pneumonia, Tuberculosis, common cold, Herpes, Hepatitis B etc. are some of the reported diseases affecting dental personnel as a result of exposure to contaminated aerosols [12].

Variations in the components of aerosol occur with the operative site and patient and can include blood, plaque/calculus, saliva, nasopharyngeal secretions, tooth components and any dental material, such as abrasives for air polishing, used during the procedure [13]. Ultrasonic scalers, Dental hand pieces, air polishers and air abrasion units are the greatest aerosols producing devices [14].

Infection control is the most integral and important part of dental practice and because of this, universal precautions are recommended by the center of disease control and prevention (CDC) and different health agencies for every single patient [15].

Different procedures and materials are recommended by American dental association (ADA) and CDC for minimizing bio aerosol
contamination such as use of personal protective equipment, surfaces decontamination, dental staff immunization, dental unit water system treatment, instruments sterilization, pre-procedural rinse and use of high-volume evacuator during dental procedures [16].

Ultraviolet chambers in the ventilation system and high efficiency particulate air are also recommended for reducing contaminated aerosols [16].

Pre-procedural rinsing with effective mouth wash is one of the convenient ways by which bacterial count can be reduced in aerosols and splatters [10]. Ravleen Kaur et al reported significant reduction in aerobic colonies by about 57% when 0.2% CHX was used as pre-procedural mouth rinse [17]. Pre-procedural mouth rinsing with 0.2% CHX was more effective than essential oil and herbal mouthwash in reducing bacterial load in aerosol in a study by Shivam Yadav et al. [18]. Likewise, significant reduction in aerosol contamination was shown by 0.2% chlorhexidine gluconate (pre-procedural mouth rinse) [19].

In a study by Koduganti Rekha et al, the two test groups 0.2% chlorhexidine and herbal mouth rinse didn’t show significant difference in reducing aerosolized bacteria [20]. Gopalakrishnan et al, in their study revealed significant reduction in colony-forming units with essential oils mouth rinse as compared with chlorhexidine gluconate (0.12% w/v) mouth rinse. [10].

Hence, this study was conducted with the aim of comparing the efficacy of two mouth rinses namely Chlorhexidine 0.2% and 5% green tea mouth rinse when used as pre-procedural rinses in reducing the number of CFU in aerosol samples generated during ultrasonic scaling.

2. METHODOLOGY

It was a quasi-experimental study, conducted in Periodontology department of Ziauddin college of Dentistry, Ziauddin University, Karachi between January 2019 to August 2019 after taking approval from Research advisory committee, ethical review committee and Board of Advanced Sciences and Research (BASR) of Ziauddin University, Karachi.

The sample size was calculated using sealed envelope non inferiority sample size calculator and non-probability consecutive sampling was used for the selection of groups. 70 subjects who reported to the Department of Periodontics, Ziauddin college of dentistry (Ziauddin University, Karachi) were selected according to the following criteria:

2.1 Inclusion Criteria
- Presence of minimum 20 permanent functional teeth
- Less than 5 mm mean probing depth.
- Plaque and gingival score between 1-3

2.2 Exclusion Criteria
- Presence of any of systemic diseases
- Tobacco use (any form)
- History of periodontal treatment in previous six months
- Pregnant and lactating females
- Use of antibiotic or other drugs affecting periodontal status in the past 6 months
- Patients allergic to CHX
- Subjects with a cardiac pacemaker
- Immunocompromised subjects

Based on the above criteria 70 subjects were randomly assigned into 2 groups (35 subjects in each group).

2.3 Blood Agar Plates Position

Reference point: Mouth of the patient.
Left side of patient’s mouth at a distance of one foot.
Right side of patient’s mouth at a distance of one foot.
Behind the patient’s head at a distance of 2 feet.

Group 1: This group consists of 35 patients.
For control side: A split mouth design was used in this study; one side that is the control side (maxillary and mandibular) of subjects’ mouth was scaled by using piezoelectric ultrasonic scaler without Preprocedural rinsing. Blood agar plates were placed at specified position to collect the aerosols and splatter generated during procedure.
For test side: the other side of patient’s mouth was scaled after pre-procedural rinsing with 0.2% chlorhexidine rinse. In similar way, the produced aerosols and splatters were collected by using fresh blood agar plates.

Group 2: This group consists of 35 patients.
For control side: One side (maxillary and mandibular) of subjects’ mouth was scaled by using piezoelectric ultrasonic scaler without
Preprocedural rinsing. Blood agar plates were placed as above.

For test: the other side of patient's mouth was scaled after pre procedural rinsing with 5% green tea mouth wash. In similar way the produced aerosols and splatters were collected on blood agar plates.

Commercial preparation of 0.2% Chlorhexidine (CHX) with the brand name Corsodyl (manufactured by GSK) and locally purchased, was used in this study.

2.4 Preparation of 5% Green Tea Mouth Wash

Pharmacology laboratory of Sir Syed College of Medical Sciences was used for preparing the extract of green tea with the following protocol.

With the help of electrical mortar green tea leaves were powdered; 100 grams of powder was mixed with 500 ml of ethanol for 48 hours; After 48 hours this mixture was filtered and the sediment was removed; The remnant solution was placed in a hot air oven at 50°C for 3-4 days; After 4 days the powder of green tea extract was obtained [21]. Finally, green tea mouthwash of 5% was prepared by adding 5 g of the extract to 100 ml of distilled water and poured into bottles[22].

2.5 Sample Collection

For both groups (control and test sides), before ultrasonic scaling, 3 blood agar plates were placed at a distance of one foot away on either side of the patient’s mouth and 2 feet behind the patient’s head.

2.6 Treatment

Written informed consent were taken from patients who fulfilled the inclusion criteria. Proforma with information regarding socio demographic characteristics (age, gender, education, ethnicity, religious affiliation, marital status etc.), was filled by the principal investigator.

Gingival index (Loe and Silness), Plaque index (Silness and Loe) and mean probing depth was recorded for each patient.

The entire operatory was sterilize with the help of ultraviolet radiation. Thorough asepsis was ensured during procedure. Only one subject was treated in a day. Before each appointment, the entire operatory test area was cleaned and disinfected using ethyl alcohol (70%). Flushing of ultrasonic scaler was done with distilled water for 2 minutes before starting the treatment. Dental unit waterline tubing were flushed by 0.5% sodium hypochlorite and was allowed to stay in tubing for 10 minutes followed by water flushing to remove the unwanted biofilm from the tubing surfaces. The amount of water dispensed, the water pressure & power settings on the ultrasonic unit was identical for each subject [10].

All subjects were treated by the same operator using the same ultrasonic unit and sterile scaler inserts. Freshly uncovered blood agar plates were placed at the three above mentioned locations to collect dispersed aerosols during the experimental procedure. The agar plates were kept exposed for 30 minutes during the professional ultrasonic scaling for all groups.

2.7 Microbiological Evaluation

The samples were covered and transported immediately to Microbiology lab of Basic Medical Sciences Institute (BMSI at Jinnah Post graduate medical Centre, JPMC Karachi).

The agar plates were aerobically incubated in a bacteriological incubator at 37°C for 48 hours. The number of colonies formed were counted using colony counter unit and statistically analyzed.

2.8 Statistical Analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 17. For numerical variables like CFU count, mean and standard deviation were calculated. A p value of less than 0.05 was considered as significant.

3. RESULTS

Results were calculated at three different locations, between the two mouthwash groups. right and left side of patients mouth and behind patient’s head.

Table 1 shows comparison of CFU mean, standard deviation and p-values among control side between Chlorhexidine and Green tea mouth wash groups and it was seen that there is a significant difference in CFU count of left control side among groups (p-value=0.02) with
Mean±Std.deviation as 38.20±9.707 among 0.2% Chlorhexidine mouth wash group and 33.43±6.963 among 5% green tea mouth wash group.

Table 2 shows comparison of CFU mean, standard deviation and p-values among test side between Chlorhexidine and green tea mouth wash groups and it was seen that there is a significant difference in CFU count of right test side showing p value=0.000 with Mean±std. Deviation as 17.3±5.323 among Chlorhexidine and as 24.94±7.264 among Green tea mouth wash groups. CFU count of left test side among both groups also showed significant difference with p-value=0.04 with Mean±Std.deviation as 21.40±6.912 among chlorhexidine mouth wash group and as 24.71±6.789 among green tea mouth wash group. Another significant difference was seen in total CFU count at test side showing (p-value= 0.001) among both groups with Mean±Std.deviation as 55.89±13.41 and 66.91±13.721 among Chlorhexidine and green tea mouth wash groups respectively.

4. DISCUSSION

According to this study, sufficient amount of aerosol and splatter was ejected during ultrasonic scaling which can easily contaminate nearby surfaces.

Table 1. Comparison of CFU count mean, standard deviation and p-values among control side between chlorhexidine and green tea mouth wash groups

<table>
<thead>
<tr>
<th>Variables (n=70)</th>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>Std. deviation</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU of Right control side</td>
<td>chlorhexidine 0.2%</td>
<td>35</td>
<td>31.60</td>
<td>7.788</td>
<td>0.14</td>
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<td></td>
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<td>34.26</td>
<td>7.139</td>
<td></td>
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<tr>
<td>CFU of left control side</td>
<td>chlorhexidine 0.2%</td>
<td>35</td>
<td>38.20</td>
<td>9.707</td>
<td>0.02*</td>
</tr>
<tr>
<td></td>
<td>5% green tea mouthwash</td>
<td>35</td>
<td>33.43</td>
<td>6.963</td>
<td></td>
</tr>
<tr>
<td>CFU of Behind control side</td>
<td>chlorhexidine 0.2%</td>
<td>35</td>
<td>27.80</td>
<td>7.128</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>5% green tea mouthwash</td>
<td>35</td>
<td>25.57</td>
<td>6.482</td>
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<tr>
<td>Total CFU control side</td>
<td>chlorhexidine 0.2%</td>
<td>35</td>
<td>97.60</td>
<td>21.663</td>
<td>0.32</td>
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<td></td>
<td>5% green tea mouthwash</td>
<td>35</td>
<td>93.26</td>
<td>14.451</td>
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</tbody>
</table>

Results are presented as mean ± SD and p-value.
*Significant p-value; CFU UNIT: CFU/Plate /30 Min

Table 2. Comparison of CFU count mean, standard deviation and p-values among Test sides between chlorhexidine and green tea mouth wash groups

<table>
<thead>
<tr>
<th>Variables (n=70)</th>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>Std. deviation</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU of Right Test side</td>
<td>chlorhexidine 0.2%</td>
<td>35</td>
<td>17.31</td>
<td>5.323</td>
<td>0.000*</td>
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<tr>
<td></td>
<td>5% green tea mouthwash</td>
<td>35</td>
<td>24.94</td>
<td>7.264</td>
<td></td>
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<tr>
<td>CFU of left Test side</td>
<td>chlorhexidine 0.2%</td>
<td>35</td>
<td>21.40</td>
<td>6.912</td>
<td>0.04*</td>
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<tr>
<td></td>
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<td>35</td>
<td>24.71</td>
<td>6.789</td>
<td></td>
</tr>
<tr>
<td>CFU of Behind Test side</td>
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<td>35</td>
<td>16.69</td>
<td>4.464</td>
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<td>17.77</td>
<td>6.436</td>
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<tr>
<td>Total CFU Test side</td>
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<td>35</td>
<td>55.89</td>
<td>13.341</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>5% green tea mouthwash</td>
<td>35</td>
<td>66.91</td>
<td>13.721</td>
<td></td>
</tr>
</tbody>
</table>

Results are presented as mean ± SD and p-value.
*Significant p-value; CFU UNIT: CFU/Plate /30 Min
The present study was conducted with the aim of evaluating the efficacy of two mouth rinses in reducing CFU count when used as Preprocedural mouth rinses before ultrasonic scaling. The present study showed a significant difference when comparing the CFU count of control side and test side in both mouth wash groups, that is mouth rinsing side in both groups showed more reduction of CFU than the non-rinsing side. This study finding was consistent with the study done by Reddy et al in which he reported that Preprocedural mouth rinsing with plaque control agents helps in reducing more CFU in aerosol samples as compared to rinsing with water only [23].

The present study showed a significant difference in reducing CFU count in both mouth rinse groups Chlorhexidine 0.2% and 5% green tea mouthwash when used as a pre procedural mouth rinse. As in our study 0.2% chlorhexidine was found to be more effective in reducing bacterial load (CFU count) as compared to green tea mouthwash.

Our findings were consistent with the study of Ammu et al., reporting Chlorhexidine more effective in reducing bacterial load in aerosol as compared to herbal mouthwash. The suggested reason by Ammu et al. for greater efficacy of Chlorhexidine (CHX) is its better penetration capacity in dental plaque [24].

In a study done by Narayana et al, it was reported that use of CHX (0.12%) Preprocedural rinse alone can be used to minimize the contaminated bio aerosols to a certain accepted level [16].

Preprocedural mouth rinsing using Chlorhexidine 0.2% along with high volume evacuator attachment is found to be very effective in reducing the bacterial load in aerosols in a study by Devker et al. [3] Greater reduction in aerosol contamination during ultrasonic scaling was reported by Narayana et al when using both, Preprocedural mouth rinsing and HVE rather than any single protocol [16].

Study reported by Yadav et al reported that CHX 0.2% was highly effective in inhibiting sub gingival plaque formation and hence prevents development of gingivitis [18]. In accordance with our study, Shamila et al reported that Chlorhexidine mouthwash was more effective than herbal mouth rinse in reducing microbial load of aerosols [25]. Similarly, study reported by Sethi et al found much reduction in CFU count with the 0.2% Chlorhexidine mouth wash as compared to herbal mouthwash [26].

Hasanah et al. also reported that Chlorhexidine rinses were more effective than tea tree oil when used as pre procedural rinse in reducing aerosol contamination [27].

Against some of the selected bacterial species, higher levels of antimicrobial action were seen with Chlorhexidine mouthwash as compared to herbal mouthwash in a study by Pathan et al. [28].

As in our study, green tea mouthwash also succeeded in reducing the CFU count significantly when comparing between the control and test sides but not more than CHX, so our study findings were consistent with the study done by Thomas et al, in which he reported that greater antibacterial activity against S. mutants and Lactobacilli spp was shown by green tea mouthwash along with some anti-fungal activity against C. albicans [29].

Thomas et al reported that on comparison of the anti-microbial efficacy of green tea mouth rinse to that of 0.2% CHX, it was found that green tea was significantly better against S. mutans, but less effective against Lactobacilli spp. and comparable against C. albicans. According to Thomas et al study, there is a paucity of reports comparing the anti-bacterial effect of green tea and CHX mouth rinses [29]. Thomas et al reported that the green tea mouth rinse was widely accepted by the study participants, whereas CHX mouth rinse had poor acceptance rate and this is a documented drawback of CHX [29].

For reducing post-operative complications of third molar surgery, study by Eshghpour et al., recommended daily rinsing with green tea mouthwash [21].

According to the study reported by Jenabian et al, the beneficial effect of 5% green tea mouth wash was seen in improving inflammatory periodontal indices after five weeks of treatment and this study supported that the daily consumption of green tea mouthwash may be beneficial to cure or prevent gingival inflammation [30].

In contrast to results of our study, the study done by Gopalakrishnan et al found that the group that
had rinsed with essential oils mouth rinse had significantly reduced colony-forming units when compared with the group that had rinsed with Chlorhexidine mouth rinse [P < 0.001][10].

5. CONCLUSION
The purpose of this study was to compare the efficacy of two mouthwashes when used as preprocedural rinses in reducing the level of viable bacteria generated in the aerosol during ultrasonic scaling. Seventy subjects with plaque and gingival score ranging between 1 and 3 were selected and were divided into two equal groups. The first group used 0.2% Chlorhexidine gluconate preprocedural rinse for one minute, while the second group rinsed with 5% green tea mouth rinse for a minute.

The results of this study confirmed that preprocedural mouth rinsing serves the purpose of reducing contamination in aerosols and splatters, that subsequently can contaminate operatory personnel. Though aerosol production cannot be totally eradicated with infection control procedures, the hazards of these aerosols can be minimized by preprocedural rinsing. The results of this study confirmed that Pre-rinsing with 0.2% chlorhexidine gluconate was more effective in reducing the aerosol contamination as compared to 5% green tea mouth rinse.

CONSENT
Written informed consent was taken from every patient.

ETHICAL APPROVAL
Ethical approval was obtained from Ethics review committee of Ziauddin University.

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COMPETING INTERESTS
Authors have declared that no competing interests exist.

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