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RESEARCH ARTICLE

EVALUATION OF INDIVIDUAL AND COMBINED EFFECT OF TEMPERED PRE-PROCEDURAL MOUTH RINSE AND MAINTAINING THE MINIMUM REQUIRED DISTANCE BETWEEN TWO CONSECUTIVE DENTAL CHAIRS IN MINIMIZING AEROSOL CONTAMINATION PRODUCED BY USING ULTRASONIC SCALAR IN PATIENTS WITH CHRONIC PERIODONTITIS

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ABSTRACT

Background: Aerosols, produced during ultrasonic instrumentation during a dental procedure are contaminative and should be assessed and controlled qualitatively and quantitatively. It is well known that pre-procedural 0.2% chlorhexidine rinse will reduce microbial count in an aerosol. But very little is known about maintaining the safest distance between two dental chairs, the efficacy of tempered (45ºC) chlorhexidine, and the combined effect of these two protocols in effective reduction of aerosols spread in a dental operating room.

Aims: To investigate the individual and combined effect of tempered chlorhexidine (45 ºC) as a pre-procedural mouth rinse and maintaining the safest distance between two consecutive dental chairs for reducing aerosol contamination produced by an ultrasonic scalar.

Materials and Methods: Thirty patients were randomly divided into 3 groups (Test I, Test II, and placebo) of 10 patients each to be administered with tempered chlorhexidine, non-tempered chlorhexidine, sterile water respectively, as a pre-procedural mouth rinse for 60 seconds. In all groups the aerosol contamination by the ultrasonic scalar was collected at 2 feet, 4 feet and 6 feet positions at 3'O clock positions on blood agar plates incubated 37ºC for 48 hours. Colony forming units (CFUs) were counted. Standardization of microbial load was done by checking pre-procedural operating room and salivary sample CFUs.

Statistical Analysis: Paired t-test for mean CFUs comparisons within groups and one-way ANOVA with Post-hoc Turkey HSD test for comparing mean differences among groups. p value ≤ 0.05 was taken as significant.

Results: Mean CFUs in Test 1 group and Test II group were significantly reduced when compared to placebo group at all distances. Also, CFU in Test 1 group was significantly reduced when compared to Test II group ($P<0.001$). Blood agar plates at 6 feet distance shown significantly less mean CFUs compared to 4 feet and 2 feet distance in all three groups. CFUs reduction below suggested levels was also found at 4 feet distance with tempered chlorhexidine.

Conclusion: Safest distance between two consecutive dental chairs is 6 feet for minimal cross infection. Still, this distance can be reduced to 4 feet if tempered Chlorhexidine is used as the pre-procedural mouth rinse. Tempered chlorhexidine is effective compared to its non-tempered counterpart. Synergistic effect of tempered chlorhexidine and maintaining safe chair distance effectively controlled aerosol qualitatively and quantitatively.

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INTRODUCTION

Airborne infection transmission through aerosol and splatter is the main concern in the field of dentistry while using ultrasonic and high-speed rotary instruments. This may hamper the health of other patients, clinician, and assistants. Micik *et al*. (1969) proposed the terminology and did most of the research work in this regard. Aerosols (less than 50 micrometers) and splatter (greater than 50 micrometers) differ in size of particles and are the main carriers of the microbial load. Splatter settle very fast but aerosol travel some distance. Due to small particle size, aerosol settle at longer distances compared to splatter. (Catuna, 1953) Aerosols with a very minute particle in the size of 0.5um to 10um in diameter have the tendency of settling in even very narrow diametric respiratory passages and causes potential respiratory tract infections. (Konig *et al*., 2002; Trenter and Walmsley, 2003) Bacteria like Pseudomonas, Mycobacterium

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and Legionella species, virus like Herpes simplex virus (HSV) and fungi like Candida species have been identified in aerosols produced from dental biofilms. (Flotra, 1973) The total bacterial count is also directly proportional to a number of clinical working hours with the ultrasonic instruments (Williams *et al*., 1973; Holbrook *et al*., 1978). It is well known that aerosol contamination can be controlled by different chemical methods like pre-procedural mouth rinses (eg. Chlorhexidine Gluoconate 0.2%), cleaning of suction lines at the end of the day with ammonia or enzymatic detergent with water (Sagar Abichandani *et al*., 2012), mechanical methods like proper ventilation and high vacuum suction devices, rubber dam usage during conservative procedures (Maria *et al*., 2008) usage of "aerosol reduction device" with ultrasonic scalars (Harrel *et al*., 1998) flushing of water from the handpiece for 30sec to 1 minute everyday morning before any procedure. (Liaqat *et al*., 2008; Caroline *et al*., 1998) were proposed methods to decrease the aerosol contamination. Though few previous studies researched about usage of pre-procedural chlorhexidine mouth rinse with different temperatures, superiority of tempered chlorhexidine over non-tempered counterpart to reduce microbial load in aerosol. (Bonesvoll *et al*,1998; Reddy *et al*, 2012, Konig *et al* 2002), and the minimum required chair distance to prevent aerosol spread very less is known about the combined effect of these two procedures in reducing aerosol contamination quantitatively.(Miller RL *et al*,1970; Chiramana S *et al*, 2013)

Therefore, the aim of the present study was to investigate the individual and combined effect of tempered chlorhexidine as the pre-procedural mouth rinse and maintaining the minimum inter-chair distance between two consecutive dental chairs for reducing aerosol contamination produced by an ultrasonic scalar.

MATERIALS AND METHODS

This was an in-vivo study, conducted in the Department of Periodontics in Sri Rajiv Gandhi College of Dental Sciences & Hospital, Bangalore in the period of July 2014-September, 2015. Informed consent participating in the study was obtained from patients before starting the study.

Inclusion criteria: Patients with moderate to severe chronic periodontitis with the age range of 25-45 years and both genders were included in this study.

Exclusion criteria: Patients with systemic diseases like respiratory diseases, using systemic antibiotics, pregnancy, and lactating mothers, mild periodontitis or gingivitis and moderate to severe periodontitis who underwent periodontal therapy in past 6 months were been excluded from the study. A total of 79 participants (45 male and 34 female) were enrolled in the study who visited the department. 16 patients were excluded from the study based on inclusion and exclusion criteria. Again, 3 more patients were excluded after incubation of samples because of large variations in CFUs count with baseline samples. Hence, 63 patients were included in the study initially to maintain the power of the study at 80%. Participation was purely voluntary. The procedures followed were in accordance with the ethical standards of the responsible institutional ethical committee and review board (IRB No: SRGCDS/2015/13-353) on human experimentation and with the Helsinki Declaration of 1975 that was revised in 2013.

Participants (n=63, male=36, female=27) were distributed randomly by using shuffling card method into three groups as Test group 1, Test group 2, and Placebo group (Figure 1). Test group 1, containing 21 patients, who rinsed with tempered 0.2% chlorhexidine (heated to the constant temperature of 45ºC with an electric kettle with temperature regulations) for 1 minute. Test group 2, containing 21 patients, who rinsed with 0.2% non-tempered chlorhexidine for 1 minute. Placebo group contained 21 patients, who rinsed with sterile water for 1 minute.

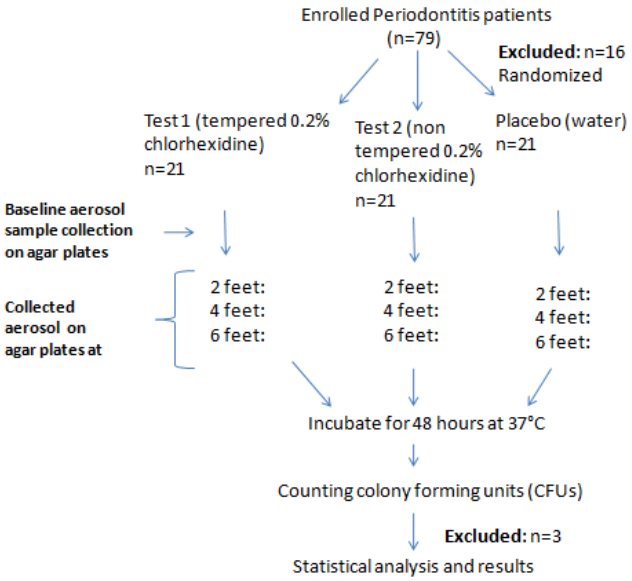

Figure 1. Flow diagram of study design

For each patient, two separate pre-sterilized rooms were been used for our research purpose. Every day morning, operating rooms were been sterilized before any procedure. In one of the rooms, a total of four samples were been collected, one is the pre-procedural sample from the operating room and other three to detect the patient salivary microbial load, collected at 2 feet, 4 feet, and 6 feet at 3' O clock position. Even in other room, four samples were been collected, one as the pre-procedural sample for operating room microbial load and other three samples were collected during respective procedures at specified distances and angulation for the same patient. Pre-procedural samples were been considered as baseline samples. Patients who would show high baseline CFUs or/and high variation in pre-procedural salivary microbial load, after incubation, were proposed to be excluded from the study. Pre–procedural Samples were been recorded on 5% sheep blood agar plates (HiMedia.MP144). Then, the patients were been instructed to rinse mouth with respective mouth rinses for 1 minute that were allotted before. Ultrasonic scaling performed in separate rooms and samples collected at specified distances (2 feet, 4 feet, and 6 feet) and angulation (3'O clock position) on 5% sheep blood agar plates (HiMedia.MP144.) on exposing agar plates for one minute and then incubated at 37°C for 48 hours. After specified period of incubation, agar plates inspected for Colony forming units (CFUs) (Figure 2, figure 3 and figure 4) and counted using pen and a click-counter method. Three patients were excluded from the study because of high pre-procedural salivary microbial load (356 CFUs, 412 CFUs, and 400 CFUs). So for final sample size of 60 patients with 20 patients in each group statistical analysis was done.

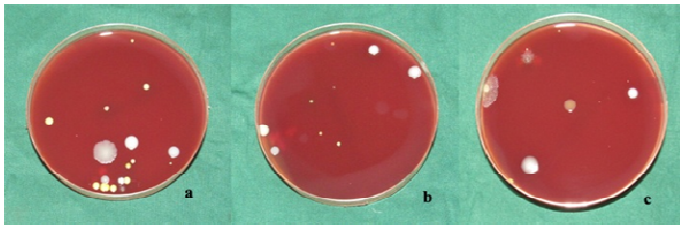

Figure 2. Agar plates showing CFUs in Test group 1, a: 2 feet distance, b: 4 feet distance, c: 6 feet distance

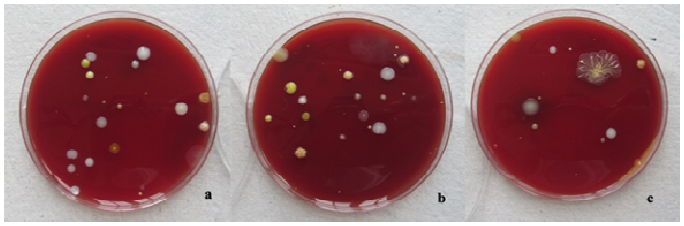

Figure 3. Agar plates showing CFUs in Test group 2, a: 2 feet distance, b: 4 feet distance, c: 6 feet distance

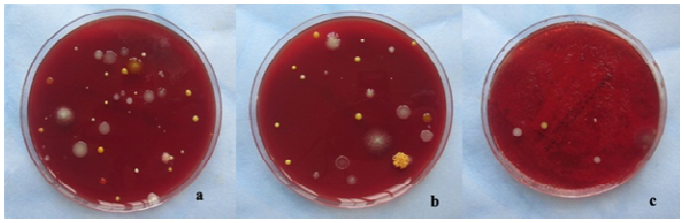

Figure 4. Agar plates showing CFUs in Placebo group, a: 2 feet distance, b: 4 feet distance, c: 6 feet distance

Statistical analysis: Statistical analysis was done with Paired t-test for comparisons within the groups and with One-way ANOVA with posthoc Turkey HSD test for comparing of mean differences in CFUs among three groups and Paired 't' test to compare mean differences of CFUs among three different distances. p value ≤0.05 was considered as significant.

RESULTS

In this study results, Table 1 shows the comparisons among mean CFU values (pre and post Pre-procedural rinse) within the groups using paired 't' test. The highly significant difference (<0.001) in mean CFU count was found in test 1 and test 2 group, while no significant difference was observed in the placebo group at all distances. In all groups, mean CFU count reduced with distance irrespective of usage of pre-procedural rinse. In all three groups, at 6 feet distance, mean CFU count was lesser than safety CFU count proposed (<35 CFU/m^3). Even in test 1 group, at 4 feet distance, the mean CFU value was within safety CFU value range. Table 2 shows, the difference between the mean CFU counts before and after the pre-procedural mouth rinse and also a comparison of these mean differences among three groups (One-way ANOVA with post-hoc Tukey HSD test) and also among three different distances (Paired 't' test). A significant difference was observed between mean differences in test 1 vs. test 2 groups (p=0.020) and test 2 vs. placebo groups (p=0.002). Comparison in mean difference between other groups at different distances showed highly significant results (p<0.001). The significant mean difference in CFU count was seen with 2 feet vs. 4 feet in both test 1 and test 2 groups (p<0.022 and p<0.018 respectively) and no significant differences were seen with the placebo group. 2feet vs. 6 feet and 4 feet vs.6 feet comparisons gave significant results in only test 1 group (p=0.004 and p=0.010 respectively), no significant results were seen in test 2 group and placebo group.

DISCUSSION

According to space zones classified by Hall (1966), which people (consciously or unconsciously) maintain from each other during common social activities as intimate (0-45 cm), personnel (60-120 cm), social (1.2-3 m), and public (over 3 m), distance always played a significant role social relations. The same is applicable to dental sciences; dentist should maintain the safest distance between two consecutive dental chairs to prevent cross contamination by aerosols. (Williams *et al*., 1970) Several types of research were been done to evaluate the maximum extent of aerosols spread by an ultrasonic instrument. Miller *et al*. 1966 investigated the maximum extent of spread of aerosol and spatter from the patient mouth by using agar plates during air-rotor usage in patient mouth, concluded that beyond 1-1.5 meters distance, the aerosol spread is very minimal. However, this study emphasized more about splatter. Study done by Chiramana *et al*. 2013 using airotor handpiece for production of aerosols; acid and litmus papers to check spread of aerosol contamination instead of agar plates, evaluated till 10 feet distance with litmus paper at

Table 1. Comparison among pre and post oral rinse usage of three groups at specific distances

Feet	Test1			Test2			Placebo		
	Pre `	Post Mean ± SD	Pre vs Post#	Pre Mean ± SD	Post Mean ± SD	Pre vs Post#	Pre Mean ± SD	Post Mean ± SD	Pre vs Post#
2 feet	155.20 ± 2.15	72.00 ± 5.74	<0.001**	154.40 ± 5.64	89.70 ± 4.90	<0.001**	148.20 ± 9.82	147.20 ± 9.94	0.453^{NS}
4 feet	103.20 ± 3.01	25.40 ± 4.22	<0.001**	106.80 ± 5.27	46.80 ± 4.13	<0.001**	107.40 ± 6.11	106.00 ± 4.81	0.173^{NS}
6 feet	33.40 ± 2.99	4.60 ± 6.33	<0.001**	32.80 ± 1.93	11.80 ± 4.26	<0.001**	34.80 ± 3.55	31.60 ± 6.02	0.333^{NS}

#Paired 't' test ; NS: p > 0.05; Not significant; **p<0.001; Highly significant

Table 2. Comparison of mean difference of CFUs count of Pre and Post oral rinse usage among all groups at specific distances

Feet	Test 1 Mean ± SD	Test 2 Mean ± SD	Placebo Mean ± SD	Test1 vs Test2#	Test1 vs Placebo#	Test2 vs Placebo#
2 feet	83.20 ± 6.48	64.70 ± 4.47	2.00 ± 4.03	<0.001**	<0.001**	<0.001**
4 feet	77.80 ± 3.19	60.00 ± 3.13	1.40 ± 2.99	<0.001**	<0.001**	<0.001**
6 feet	68.80 ± 7.55	61.00 ± 3.80	3.20 ± 6.13	0.020*	<0.001**	0.002*
2 feet vs 4 feet$	0.022*	0.018*	0.811			
2 feet vs 6 feet$	0.004*	0.124	0.469			
4 feet vs 6 feet$	0.010*	0.521	0.475			

#One-way ANOVA with post-hoc Tukey HSD; $ Paired 't' test; *p<0.05; Significant; **p<0.001; Highly significant

every 1 foot interval to detect aerosol contamination and concluded that 6 feet is the maximum distance aerosol can travel and that could be the safest distance between two consecutive dental chairs. Even in our study, there was significant reduction in aerosol contamination at 6 feet distance in all three groups, test group 1, test group 2, and placebo group (4.60 ± 6.33CFUs, 11.80 ± 4.26 CFUs, and 31.60 ± 6.02 CFUs respectively) which are similar to the previous studies. Usage of different concentrations of Chlorhexidine as the pre-procedural mouth rinse for 60 seconds for reducing bacterial load in aerosol was been well proven (Bhanu *et al*., 2011). Bonesvoll *et al*, 1974 investigated the influence of concentration, time, temperature and pH of 0.2% Chlorhexidine rinse in the oral cavity. The investigation concluded that, no significant increase in substantivity of the parent chemical in the oral cavity on raising the temperature from 22°C to 60°C. Though the rate of chemical reaction was said to have increased with increase in temperature, it was shown that tempered Chlorhexidine has a more antimicrobial effect than the non-tempered counterpart but exact mechanism was not proposed. Reddy *et al*., 2012; Konig *et al*., 2002 further investigated the effect of tempered chlorhexidine as pre-procedural mouth rinse by heating chlorhexidine to 47ºC; and concluded that tempered chlorhexidine is more effective compared to non-tempered chlorhexidine. But, Green B G, 1985 proposed that 46.0 ºC is the maximum temperature where oral and peri-oral structures will not get damaged irreversibly. Because of this reason, in our study, we have limited tempering chlorhexidine till 45 ºC for safe side usage. Nevertheless, our study also shows similar results as previous studies. Tempered chlorhexidine gave the significant reduction in aerosol contamination compared to nontempered counterpart at all three distances, 2feet, 4 feet, and 6 feet distances ($p<0.001$, $p<0.001$ and $p<0.020$ respectively).

The quality of air around the surgical area is very important for prevention of cross contamination of airborne and blood borne infections. According to Italian Institute for Occupational Safety limits (ISPESL) and the International Standard Organization (ISO) standards, acceptable levels of air microbial contamination in operating theaters should be in the range of 35 CFU/m^3 to 180 CFU/m^3 (Spagnolo *et al*., 2013). Bali R and his co-workers (2012), suggests that ideal level of microbial load in empty operation theater should not exceed 35 CFU/m^3. In this current study, we have taken pre-procedural samples from empty operating rooms and salivary samples of the patients, proposed to exclude all samples of a patient if his/her pre-procedural sample CFU is greater than standardized levels after incubation. We have included patients with 140-200 CFUs at 2 feet, 80-140 CFUs at 4 feet, and 20-80 CFUs at 6 feet distance as standardization. Also, <35 CFU/m^3 in pre-procedural operating room microbial load was taken as standardization. Since very high and/or very low scores can impact the mean values, we have excluded patients with extreme pre-procedural sample values. We got three patients with such salivary microbial loads, these three patients were excluded after incubation of agar plates. In this current study, the mean number of CFUs found as 33.40 ± 2.99 (Pre-Op) and 4.60 ± 6.33 (Post-Op) with tempered chlorhexidine at 6 feet distance, which was below the acceptable levels suggested by ISPESL and ISO. Even with test group 2 and placebo group, mean CFU values (both pre-op and post-op) were below acceptable levels at 6 feet distance; suggestive of 6 feet to be the safest distance to maintain CFUs in acceptable level. Usage of tempered chlorhexidine with maintaining 6 feet distance reduced mean aerosol to significant level suggestive of the combined effect of these two protocols. Even 4 feet distance was shown as the safest distance in test 1 group with mean CFUs in the acceptable range (25.40 ± 4.22) and show efficacy of tempered chlorhexidine over non-tempered counterpart. Comparison of mean difference in CFUs with test 1 vs. test 2 at all distances gave significant results, suggestive of tempered chlorhexidine to be effective in reduction of quality of aerosol compared to its non-tempered counterpart. Mean CFUs at 2 feet distance is higher than acceptable levels in all three groups i.e., test 1, test 2 and placebo groups (72.00 ± 5.74, 89.70 ± 4.90 and 147.20 ± 9.94 respectively) even after pre-procedural rinses, this suggests, dental professionals and their assistants has to follow extra barrier protocols to prevent aerosol contamination.

Strengths of this study

We have taken pre-procedural samples of the saliva so that we can standardize the samples of the patient's saliva excluded three patient. A great variation in CFUs any patients salivary sample may change mean values of the CFUs count of the study. We have also checked pre-procedural operating room CFUs count for baseline values for standardization.

Drawbacks of study

The assessment of the extent of the spread of the aerosols in this study was not done under various environmental conditions, i.e. with fan, without Fan, with A/C and without A/C, high vacuum suction usage, cross ventilation, which usually influences the extent of spread of airborne particles. The quality of microbiota was not assessed in this study.

Conclusion

This research signifies the prevention of aerosol contamination is very important personally and professionally. However, in practice, it is impossible to achieve total elimination of bacterial aerosols during dental treatment. The safest distance between two consecutive dental chairs is 6 feet for minimal cross infection. Further, this distance can be reduced to 4 feet, if tempered Chlorhexidine is used as the pre-procedural mouth rinse. Tempered chlorhexidine is effective compared to its non-tempered counterpart. Synergistic effect of tempered chlorhexidine and maintaining safe chair distance effectively controlled aerosol qualitatively and quantitatively. Hence, the combination of some protocols like pre-procedural mouth rinse and maintaining a safe range of distance around a dental chair would at least minimize the airborne cross contamination.

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Competing interests: None

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