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Research Article

THE EFFICACY OF PREPROCEDURAL MOUTH RINSE OF 0.2% CHLORHEXIDINE AND COMMERCIALLY AVAILABLE HERBAL MOUTH CONTAINING SALVADORA PERSICA IN REDUCING THE BACTERIAL LOAD IN SALIVA AND AEROSOL PRODUCED DURING SCALING

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ABSTRACT

Aim: This study is conducted to evaluate the efficacy of pre procedural mouth rinses, in reducing the bacterial count in the oral cavity which in turn could reduce the risk in generating aerosol.

Objectives

- To compare the effectiveness of herbal mouthwash as a pre procedural mouth rinse over 0.2% chlorhexidine mouthwash in reducing the bacterial count in saliva.
- To determine the extent of the spread and to assess the amount of contamination in the clinical environment by collecting the aerosol at 1 foot, 2 feet and 3 feet distance after the pre procedural mouth rinsing with herbal mouthwash and 0.2% chlorhexidine mouthwash.
- To know whether the reduction of bacterial count in the saliva has any effect on the expected proportionate reduction of the aerosol production in the two groups of patient with herbal and chlorhexidine mouthwash.

Material and methods: A total of 30 patients were selected and randomly divided into three groups. Group I consists of 10 patients who rinsed with normal saline for 60 seconds. Group II consists of 10 patients who rinsed with 0.2% chlorhexidine mouthwash for 60 seconds. Group III consists of 10 patients who rinsed with herbal mouthwash for 60 seconds. Salivary sample were collected in sterile disposable container before the mouth rinse and 5 minutes after the mouth rinsing. The samples were subjected to bacteriological analysis to estimate the total bacterial count. Aerosols produced during the oral prophylaxis procedure were collected on BHI agar plates by exposing the plates at 1 foot, 2 feet and 3 feet distance and the exposed plates were incubated at 37°c aerobically for 24 hours. The number of colony forming units (CFU) in aerosol and CFU in the saliva were counted and statistically analyzed. Care was taken to exclude the bacterial colonies that were of non oral origin.

Results: Reduction in the bacterial load using 0.2% of chlorhexidine gluconate mouthwash is found to be significant in both saliva and aerosol produced during scaling. Although the CFU is reduced to 99.91% in saliva, the reduction is not proportionate in the aerosol produced among the same group.

Conclusion: Chlorhexidine mouthwash as a pre procedural rinse is more effective than the herbal mouthwash. Preprocedural rinse has a definite benefit in the treatment perspective but as aimed, the reduction in the bacterial load in saliva has not proportionately decrease the bacterial load in the aerosol.

Keywords: Dental aerosol, Splatter, Pre procedural mouth rinse, Chlorhexidine mouthwash, Herbal mouthwash, Dental plaque, Bacterial load in aerosol, Bacterial load in saliva.

INTRODUCTION

Occupational hazards are dangers to human health and well being which are associated with specific occupations. Although efforts are made to reduce these health hazards, they are unavoidable in the work place by nature of the profession. Occupational health risk is being monitored and documented in dental profession since late 1920s [1]. Many studies have been reported with incidence of infection more in dentist compared to patient who visited the clinic. Mosley JW et al demonstrated that, a large number of dental students in US were reported with exposure to hepatitis however there are also cases reported with transmission of human immunodeficiency virus (HIV) from a dentist to five of his patients [2].

Most of the procedures carried out in the mouth, results in the formation of aerosol and splatters which are commonly mixed with bacteria, fungi, protozoa and even blood borne viruses [3,4]. The terminology, "aerosol and splatter" in dental environment were proposed by Micik in their pioneering work on aerobiology [5]. Aerosols are combination of both liquid and solid particles. Majority of the particles in the aerosol 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 [3,6,7]. Microorganisms that are present in the mouth and respiratory tract can be transported in the aerosol produced during dental procedures leading to respiratory infections, skin infection and other systemic diseases in immunocompromised patient. They also contaminate the mucous membrane of the mouth, respiratory passages, eyes of dental professionals and patients and the surrounding surfaces [3]. Besides airborne infection, it should also be understood that, aerosol is a vehicles that can even transmit blood borne viruses to the people at close vicinity of the patient.

Procedures like hand scaling and the use of air water syringes during dental treatment generates large droplet which is known as splatter. This is controlled by standard barriers like gloves, masks and eye protections however certain instruments like high speed handpieces and ultrasonic scaler tips, produces small droplets of aerosol which is highly contaminated leading to raised risk towards airborne transmission of infection [6]. Sources of dental aerosol produced during dental procedures are from the patient, dental unit waterlines (DUWL) and instruments used [8]. The bacteria present

in the aerosol, whose source is the patient, originates from the saliva as well as the dental plaque.

As a routine, precautionary methods are carried out in dentistry to reduce the external contamination during ultrasonic scaling like flushing of water from the handpiece at the start of each clinical day to reduce microbial accumulation due to overnight waterline stagnation and between patients for 30 seconds to 1 minute [4]. High vacuum suction/evacuator [5] and use of rubber dam during conservative procedures [7] also helps in reducing external contamination. At the end of the day, the suction lines should be cleaned with ammonia or enzymatic detergent with water [9] and the use of pre procedural mouth rinses which aids in reducing aerosol contamination.

Preprocedural rinsing using 0.2% chlorhexine gluconate for duration of 60 seconds can cause substantial reduction in bacterial counts [10]. Study conducted by Snophia S et al in 2011 has also proved chlorhexidine as an effective primary measure in reducing aerosol cross contamination when using dental devices in a dental set up [6]. Hence the present study was conducted to compare the effectiveness of herbal mouthwash as a pre procedural mouth rinse over 0.2% chlorhexidine mouthwash in reducing the bacterial count in saliva and also to know whether the reduction of bacterial count in the saliva has any affect on the proportionate reduction of the aerosol production in two groups of patient with herbal mouthwash and chlorhexidine mouthwash.

MATERIALS AND METHODS

MATERIALS

- Saliva samples from patients
- 0.2% chlorhexidine gluconate
- Herbal mouth wash
- Normal saline
- Brain Heart Infusion agar
- Sterile disposable container
- Disposable petri dishes (100mm)
- 5ml and 2ml syringes

METHODOLOGY

This study was conducted in Saveetha Dental Hospital, Chennai, Tamilnadu, India. A total of 30 subjects whose age group was within 18 to 50 years were selected for the study. The patients were randomly allocated in 3 groups after the estimation of the plaque index. One way ANOVA was used to statistically compare the mean plaque index between the groups. Each group consists of 10 subjects.

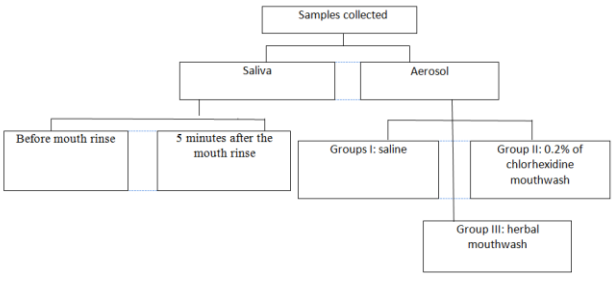

Figure 1: It shows the diagrammatic depiction of the study design

Inclusion criteria

- Minimum of 20 healthy permanent tooth
- Absence of any dental treatment for the past 6 months
- Patient with plaque and gingival index score within 1 and 3

Exclusion criteria

- History of any systemic disease
- Pregnant women
- Patients who is on any antibiotics for the past 24 hours
- Patient who were not in a habit of using chemical mouthwash

Groups

- Group I : This group consist of 10 patient who is given rinse with normal saline for 60 seconds
- Group II : This group consist of 10 patient who is given rinse with 0.2% chlorhexidine for 60 seconds
- Group III : This group consist of 10 patient who is given rinse with herbal mouthwash for 60 seconds

STEP 1 : To estimate the bacterial load in saliva

Collecting salivary sample for bacteriological count before and after mouth rinse

Before starting the ultrasonic scaling, in all the 3 groups, salivary sample was collected in a labeled sterile disposable container. According to the group, patients in group I were asked to rinse with normal saline, in group II with 15ml of 0.2% of chlorhexidine gluconate and in group III with 15ml of herbal mouthwash for 60 seconds. After 5 minutes, salivary sample were collected again from each patients. All the samples collected were diluted with normal saline at the ratio of 1 in 10 which is 0.5ml of saliva in 4.5 ml of normal saline in a test tube. The test tubes were labeled and shaken properly to get a uniform mixture of the salivary sample and saline. Using a calibrated inoculating loop, the sample is innoculated on the Brain Heart Infusion (BHI) agar plates to obtain isolated colonies. All the agar plates were incubated for 24 hours at 37°c aerobically. CFU in the culture plates were counted and statistically analyzed.

STEP 2 : Estimation of the extent of the aerosol produced

Exposing the plates to estimate the amount of aerosol

Certain standardization were maintained before the ultrasonic scaling procedure, like patients were placed in supine position, use of clean water in dental unit water line, disinfected bottles were used, handpieces were flushed several minutes at the start of each day and 2 minutes before each procedure from the ultrasonic scaler tip. Care was also taken regarding the cross ventilation. The oral prophylaxis was done using a piezoelectric ultrasonic unit which comprised of a handpiece and scaler tips. The speed of the unit was set at 7 KiloHertz for all patients. High speed evacuator/ suction were used for all the procedures.

Following this, 100mm BHI agar plates were exposed on patient's chest area and the operator's side at a distance of 1 foot, 2 feet and 3 feet away from the patient's mouth. The agar plates were exposed for 30 minutes during the professional ultrasonic scaling in all the 3 groups. The agar plates were incubated for 24 hours at 37°c aerobically. The CFU were counted and statically analyzed.

RESULTS

Table 1 shows the mean rank of colony forming units on agar plates for each of the 3 groups at 3 standard locations (1 foot, 2 feet and 3 feet) away from the patient's mouth. The mean rank values between the groups at 1 foot, 2 feet and 3 feet was found to be statistically significant. The colony forming units in the culture plates obtained from saliva was compared among all the 3 groups and was found to be statistically significant (*p value = < 0.001). The results demonstrated that, even rinsing with saline and mouthwashes could reduce the microbial load in the oral cavity. (Table 1)

Table 1: It shows the comparison of the bacterial load in the aerosol and saliva for all the 3 groups

(Kruskal- Wallis Test)

Variables	Group	N	Mean Rank	P-Value
IN AEROSOL 1 FEET	I	10	21.25	0.010
	II	10	9.30	
	III	10	15.95	
IN AEROSOL 2 FEET	I	10	20.05	0.032
	II	10	9.90	

	III	10	16.55	
IN AEROSOL 3 FEET	I	10	23.30	
	II	10	8.70	0.001
	III	10	14.50	
IN SALIVA BEFORE MOUTHWASH	I	10	5.60	
	II	10	15.60	<0.001
	III	10	25.30	
IN SALIVA AFTER MOUTHWASH	I	10	15.80	
	II	10	5.50	<0.001
	III	10	25.20	

Table 2 shows the pair wise comparison of the bacterial load reduction in the aerosol and in the saliva before and after the mouth rinse. Though there is reduction in the bacterial load in all the 3 groups, the amount of reduction seen in chlorhexidine is significantly high. (Table 2)

Table 2: It show the pair wise comparison of the bacterial load reduction in the aerosol and in saliva before and after mouth rinse between all the 3 groups

(Mann Whitney U Test with Bonferroni correction)

Variables	Pairs (mouthwashes used)	P-Value
IN AEROSOL 1 FEET	SALINE vs CHLROHEXIDINE	0.007
	SALINE vs HERBAL	0.534
	CHLROHEXIDINE vs HERBAL	0.273
IN AEROSOL 2 FEET	SALINE vs CHLROHEXIDINE	0.030
	SALINE vs HERBAL	0.999
	CHLROHEXIDINE vs HERBAL	0.273
IN AEROSOL 3 FEET	SALINE vs CHLROHEXIDINE	0.001
	SALINE vs HERBAL	0.076
	CHLROHEXIDINE vs HERBAL	0.422
IN SALIVA BEFORE MOUTHWASH	SALINE vs CHLROHEXIDINE	0.030
	SALINE vs HERBAL	0.001
	CHLROHEXIDINE vs HERBAL	0.037
IN SALIVA AFTER MOUTHWASH	SALINE vs CHLROHEXIDINE	0.026
	SALINE vs HERBAL	0.049
	CHLROHEXIDINE vs HERBAL	0.001

Table 3 shows the percentage change of CFU in saliva before and after using mouthwashes in all the 3 groups. Both chlorhexidine and herbal mouthwash could demonstrate a reduction in the microbial load in saliva (*p= <0.05). (Table 3)

Table 3: It shows the significance of the reduction in the bacterial load in the saliva for all the 3 groups

Mouthwash group	P-Value
GROUP I: SALINE	0.061
GROUP II: CHLROHEXIDINE	0.005
GROUP III: HERBAL	0.005

The pair wise comparison for the percentage change in all the 3 groups was statistically analyzed. At all location, the reduction of CFU was statistically significant in both chlorhexidine and herbal mouthwash keeping the data collected for saline mouth rinsing as the base line value.

DISCUSSION

Aerosol produced in the dental environment is highly contaminated with bacteria and can pose a potential threat to the dentist as well as the patient. A study done by Szymańska J et al in 2007 demonstrated that ultrasonic unit produces the greatest amount of aerosol and splatter in dentistry [3]. Several other studies also show that this procedure is one on the greatest airborne contaminant in dentistry [11,12]. Hence the present study was conducted to assess the aerosol contamination using an ultra sonic scaler unit and also to assess whether the dental aerosol produced are solely from the patients mouth.

Periodontitis is an inflammatory disease of the supporting tissue of the teeth caused by specific microorganisms or groups of specific microorganisms resulting in progressive destruction of the periodontal ligament and the alveolar bone with pocket formation, recession or both [13]. Dental plaque being the prime etiological agent comprises complexes of microorganisms, both bacterial and viral origin in the gelatinous matrix. It gets dispersed when mechanically acted upon pressure of food, friction of soft tissues or dislodgement by the ultrasonic devices [14]. Therefore the aerosols produced by ultrasonic scaler units are heavily contaminated by microorganism which have the capability of disease transmission leading to various diseases like mild flu, pneumonia [4,15,16], streptococcal and staphylococcal infections, viral infection, conjunctivitis [8], amoebic keratitis [17], tuberculosis and severe acute respiratory syndrome (SARS). Blood borne pathogens like HIV, HBV, and HCV can be transmitted through the inhalation of blood containing aerosol via the microlesion in the mucosa of the airways which acts as the potential access for such viruses [7,18]. It can also contaminate the nearby instruments on the instrument trays which can further act as a source of infection to the patient. Hence this study was done to demonstrate the efficacy of the mouthwashes in controlling the aerosol produced during oral prophylaxis using ultrasonic scaler unit.

A study done by Gupta DG et al demonstrated the efficacy of preprocedural rinsing with chlorhexidine in reducing the aerosol contamination produced by ultrasonic scaling [19]. Use 0.2% chlorhexidine gluconate mouthwashes as a pre procedural mouth rinsing for the duration of 60 seconds can cause substantial reduction in bacterial counts [10]. Chlorhexidine is a cationic bisguanide molecule that can strongly bind to the hydroxyappetite, the organic pellicle of the tooth, oral mucosa, salivary protein and bacteria therefore it exhibits superior substantivity in oral cavity [6]. It is considered to be the most effective anti plaque and anti gingivitis agent [13]. It was found to be bacteriocidal at higher concentration and bacteriostatic at lower concentration.

Apart from chlorhexidine, other mouthwashes containing herbal products which have anti plaque efficacies are also being marketed. AM Khalessi et al in 2004 demonstrated the efficacy of herbal mouth wash in controlling the plaque formation [20]. Some of the main ingredient which is present in the herbal mouthwash used in this study is Salvadora persica, Terminalia bellerica, Piper betel and etc. These ingredients have anti bacterial property and also help in the releases of calcium and fluoride into saliva [21]. Since dental plaque comprises of microorganism of both bacterial and viral in origin, herbal mouthwashes with extracts demonstrating antiviral activity like neem would be a better choice [22].

Other than intraoral sources, aerosol contamination can also occur due to cross ventilation and contaminated of water used in water pipe lines. Investigators have reported contamination from the dental unit water line (DUWL) due to narrow bore water lines, water stagnation, heating of dental chair units and contamination of reservoir water [23]. Therefore in this study, disinfected bottles with clean water were used during the dental procedure. Flushing of water was done for 2 minutes before each procedure along with the usage of high speed evacuator. This was in accordance to the study reported by Caroline L et al in 1998 [4].

Salivary samples were collected from each patient before and after using the mouth rinse in all the 3 groups. This was done to assess whether the reduction in the bacterial load after using the mouth rinse is proportionate with the reduction in the bacterial count obtained from the aerosol. The salivary sample collected was inoculated in the BHI agar plates. BHI agar plates were also exposed on patient's chest area and the operator's side at a distance of 1 foot, 2 feet and 3 feet away from the patient's mouth. The agar plates were exposed for 30 minutes during the professional ultrasonic scaling in all the 3 groups. The agar plates were incubated for 24 hours at 37°c aerobically. The CFU were counted and statically analyzed. BHI agar plates were used because it is an enriched media and supports the growth of fastidious organisms.

The results demonstrated that the microbial load in the saliva was reduced in both chlorhexidine and herbal group (p value = <0.05). It was found that chlorhexidine mouthwash could reduce the bacterial load to 99.91% in saliva while herbal mouthwash could reduce the bacterial load to only 58.27%. Hence chlorhexidine mouthwash has better antibacterial activity than herbal mouthwash.

In aerosol, only chlorhexidine mouthwash was found to have significant reduction in the bacterial load. Pair wise comparisons between the mouthwashes were done with the saline rinsing as the base line value. It was found that at all locations, both chlorhexidine and herbal mouthwash was effective. However the percentage reduction of the bacterial load in the aerosol in chlorhexidine group was much higher in comparison with the herbal group. Therefore chlorhexidine is proved to be better than herbal mouthwash. This is in accordance to a study done by Ranjan Malhotra et al in 2011 [24]. The reason to this could be due to better penetration of chlorhexidine to dental plaque.

Although the CFU is reduced to 99.91% in saliva by using chlorhexidine mouthwash, the reduction is not proportionate in the aerosol produced among the same group. In previous studies, preprocedural rinse reduces the bacterial load in the aerosol produced. However in the current study, there is no demonstrable reduction of the bacterial load in the aerosol inspite of 99.91% reduction in the salivary count when 0.2% of chlorhexidine is used. The pre procedural mouth rinse given aimed at reducing the bacterial load in the aerosol produced should be re-evaluated.

CONCLUSION

In the present study it is found that, the preprocedural mouth rinse has a definite benefit in the treatment perspective but as aimed, the reduction of the bacterial load in the saliva has not proportionately decreased the bacterial load in the aerosol. Therefore it is concluded that the bacterial load in the aerosol originates from the saliva, plaque, dental unit water line and to some extent from the atmosphere and not only form the saliva.

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