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Position Wise Quantification of Microbial Load in Aerosols Produced During Ultrasonic Scaling

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ABSTRACT: Oral cavity harbours millions of bacteria and viruses from respiratory tract, saliva and dental plaque, which get aerosolized when they come in contact with the dental equipment, particularly the high-speed dental drills and ultrasonic scaler. These microorganisms have important impacts on air quality and can cause a serious health threat to the clinician, patients and the surroundings. Notably, the aerosols are not dispersed evenly in the operatory room, and contradictory observations are reported in various studies. In this study, the aerosol produced during ultrasonic scaling was collected on blood agar plates positioned at chest area of patients, operators and assistants, while aerosol collected in the operating room before the procedure, considered as baseline (n=80). Microbial colonies formed on the blood agar plates were counted after incubating for 24 hours at 37° C. Our observation directed maximum microbial colonies at the chest area of the operator and lowest at the chest area of the assistant. We may draw a conclusion that not only the Dental personals but also the patient is main targets of the microorganisms generated during oral procedures.

KEYWORDS: Aerosol, Pre-procedural mouth rinse, Ultrasonic scaling; Microbial colonies.

I. INTRODUCTION

Aerosols are the suspensions of solid and or liquid particles containing bacteria and viruses in the air, size being ranges from 0.001 mm to more than 100 µm[1]. Heavy droplets fall to the floor and become part of floor dust. Again, they may reduce in size with evaporation of the water molecules, referred to as 'droplet nuclei' (0.5 µm - 10 µm). These 'droplet nuclei' contain microorganisms either in non-vegetative or spore forms and remain latent in the atmosphere up to 6 days[2, 3] with greater potentiality to create health problems when it comes in contact with the skin and mucous membranes, and may also reach the respiratory passages and lungs[4,5], though it depends on the factors, like infecting dose, virulence and the host's immune system[6].

Oral environment is uniquely moist and warm, and contains certain metabolites that favour microbial growth. Thus, oral cavity harbours millions of bacteria and viruses from respiratory tract, saliva and dental plaque. These microorganisms get aerosolized when they come in contact with the dental equipment, particularly the high-speed dental drills and ultrasonic scaler[7,8]. Moreover, this equipment uses water as a coolant which is splattered during vibration of the tip and becomes contaminated when it is mixed with saliva and plaque, and subsequently acts as a major risk factor for transmission of various diseases. However, the amount of contamination of dental aerosol depends on the quality of saliva, nasal and throat secretion, blood, dental plaque, periodontal infection, and presence of any other dental infection[9]. Miller (1976) reported that aerosols generated from the subjects with periodontitis contain up to 100,000 bacteria per cubic foot of air[4]. They have significant impacts on air quality and can cause a serious health threat to the clinician, patients and the surroundings in the form of systemic conditions like common cold, tuberculosis and severe acute respiratory syndrome (SARS). In addition, blood-borne pathogens like HIV, HBV, and HCV are also transmitted through inhalation of the blood containing aerosols [10]. However, these aerosols are not dispersed evenly in the same operatory room, and greatest

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concentration has been shown within 2 feet of the patient radiating more either towards the chest of the patient or the face of the operator [2, 11-14]. Considering this fact, the present study was undertaken to determine the microbial load in aerosols by assessing the number of bacterial colonies formed in Blood Agar culture plates positioned at various distance of the working area during ultrasonic scaling.

II. MATERIALS AND METHODS

The present study was carried out in the Department of Periodontics and Oral Implantology, Regional Dental College & Hospital, Guwahati, Assam, India in collaboration with the Department of Microbiology, Gauhati Medical College, Guwahati, Assam, India. The study was carried out in accordance with the ethical guidelines of the Institutional Research and Ethical Committee.

A total of 80 subjects with periodontitis were selected from the Out Patient Department (OPD), Department of Periodontics and Oral Implantology, irrespective of sex, religion and socioeconomic status. Subjects were explained the entire procedure in details and written consent was obtained from each of them. The subjects were selected on the basis of the following criteria:

Inclusion criteria:

- Subjects of 20 to 65 years old with chronic periodontitis
- Subjects having not less than 20 permanent teeth
- Systemically healthy subjects
- Subjects received no antibiotics and dental treatment especially oral prophylaxis in last 3 months of time

Exclusion criteria:

- Pregnant and lactating mothers
- Smokers

CLINICAL PARAMETERS

A full mouth clinical examination was carried out on all the participants. The periodontal health status was recorded using Plaque Index, Probing Pocket Depth and Clinical Attachment Level.

Plaque index by Silness and Loe, 1964 (Loe, 1967)[15]:

Teeth were air dried and examined visually. A 17 Shepherds hook explorer was used to evaluate the tooth surfaces. Teeth surfaces were explored in the cervical third, near the entrance to the gingival sulcus. The scoring criteria are as follows:

- Score 0: No plaque
- Score 1: A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface.
- Score 2: Moderate accumulation of soft deposits within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye.
- Score 3: Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

Scores from all the surfaces of a tooth were added and divided by the number of teeth examined to obtain the score for per tooth. Plaque index for an individual was obtained by dividing the sum of all the individual scores by the total number of surfaces recorded

Probing pocket depth:

Probing Pocket Depth was measured using the UNC-15 periodontal probe. The working end of this probe is 15 mm long with markings at each millimeter and colour coding at 5^{th}, 10^{th} and 15^{th} mm. It was measured from the gingival margin to the base of the pocket at four points around a tooth, and those were at distofacial line angle, mesiofacial line angle and at the centre of the facial/buccal and lingual surfaces. Probing Pocket Depth for an individual was obtained by dividing the sum of all the individual scores by the total number of surfaces recorded.

Clinical Attachment Level:

Clinical attachment level was measured from cementoenamel junction (considered as reference point) to the base of the pocket using UNC-15 periodontal probe in a similar manner to that of the probing pocket depth, as mentioned above. Clinical attachment level for an individual subject was obtained by dividing the sum of all the individual scores by the total number of surfaces recorded.

MICROBIOLOGICAL PARAMETERS

Preparation of culture media:

40 grams of HIMEDIA Blood agar base was suspended with 1000 ml of distilled water in a conical flask. It was heated till boiling to dissolve the medium completely and autoclaved at 15 lbs pressure, 121° C for 15 minutes. The sterile media obtained was then cooled to 45 - 50° C. Following this, 5% sterile defibrinated sheep blood was added to the flask. It was mixed well and poured into the sterile petri plates. The prepared agar plates were stored in refrigerator at 2 - 8° C up to 5 - 6 days.

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Collection of aerosols:

To determine the number of microorganisms in the aerosols, the agar plates were placed at 4 different positions for collection of aerosols prior or during the ultrasonic scaling:

- Position 1: Operatory room, 4 feet away from the dental chair, 30 minutes prior to the ultrasonic scaling, considered as baseline count
- Position 2: Chest of the patients during ultrasonic scaling
- Position 3: Chest of the operator during ultrasonic scaling
- Position 4: Chest of the assistant during ultrasonic scaling

The agar plates were coded and positioned with the help of double sided adhesive tape during ultrasonic scaling for position 2, 3 and 4. To determine if there were any aerosolized bacteria present in the operatory room, blood agar plates were positioned in the designated area (position 1) for 30 minutes before conducting the ultrasonic scaling.

Method of colony counting:

Agar plate with microbial colonies was placed on the illuminated pad of the digital colony counter (INSIF, Haryana, India). As the colony in the agar plate was marked with the pen provided in the colony counter, an audible beep confirms the count and digital read out appears on the display.

Clinical Procedure:

The operatory room was fumigated using formaldehyde (40%) for 15 minutes on the day prior to treatment. Only one patient per day was treated to avoid aerosol contamination. Same operatory room was used for all the samples. Before each appointment, the operatory surfaces were cleaned and disinfected using 70% ethyl alcohol.

After the baseline sampling and prior to the ultrasonic scaling, coded agar plates were placed accordingly and stabilized with adhesive tape for aerosol collection, as described before. Then scaling was carried out for 30 minutes using a piezoelectric ultrasonic scaler (DTE-D5), with controlled frequency and the pressure of the water coolant was maintained at constant level. A motorized suction was used during the treatment procedure.

Immediately after the scaling, agar plates were removed and sealed. Then they were incubated at 37°C for 24 hours in an increased CO_2 chamber (BOD Incubator, Chennai, India) and colonies that grew on each plate were counted using a colony counter.

Statistical analysis:

All the data collected was analysed statistically using IBM Statistical Package for Social Sciences (SPSS) version 20. Analysis of Variance (ANOVA) tests were done to compare the means of two groups with significant inference at $p \leq 0.05$.

III. RESULT

CLINICAL PARAMETERS

The mean Plaque Index (2.17 ± 0.38), Probing Pocket Depth (3.90 ± 0.70) and Clinical Attachment Level (4.63 ± 0.78) of the participant subjects indicated that all the participants were suffering from periodontitis of equal intensity.

MICROBIOLOGICAL PARAMETERS

Position wise count of microbial colonies:

The position 1 was about 4 feet away from the dental chair in the operatory room where agar plates were placed 30 minutes prior to the ultrasonic scaling. Microbial colony count at this position was considered as the baseline. Average microbial colony count (Mean ± SD) in this position was found to be 14.91 ± 11.42 that ranged from 13.25 to 16.80 (Table 1). In position 2, where microbial colonies were counted in the agar plates placed on the chest of the patients during ultrasonic scaling, the mean number of microbial colonies was 302.95 ± 74.48 (range 105.00 to 458.00). In position 3, where microbial colonies were counted in the agar plates placed on the chest of the operator during ultrasonic scaling was 429.20 ± 62.62 (range 298.00 to 560.00). In position 4, where microbial colonies were counted in the agar plates placed on the chest of the assistant during ultrasonic scaling, the mean number of microbial colonies was 193.90 ± 87.92 (range 65.00 to 415.00). Schematic representation of the microbial colonies in all four positions of blood agar plates is shown in Figure1.

On position wise comparison, the lowest number of microbial colony was seen in the operatory room before scaling, while the highest number of microbial colony was observed in the agar plates placed on the operator's chest (position 3) followed by position 2 (patient's chest) and position 4 (assistant's chest). The numbers of colonies were observed to be 28.78-, 20.31- and 13.00- times more in position 3, 2 and 4, respectively, than that of the aerosolized bacteria in the operatory room (position 1). The differences in the mean values of microbial colonies in all the four positions were found to be statistically very highly significant ($p < 0.001$).

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Table 1: Position wise average microbial colony count

Sl No	Position of agar plate	Mean ± SD	Range
1	Baseline†	14.91 ± 11.42	13.25 - 16.80
2	On chest of patients††	302.95 ± 74.48	105.00 - 458.00
3	On chest of operator††	429.20 ± 62.62	298.00-560.00
4	On chest of assistant††	193.90 ± 87. 92	65.00 - 415.00
†4 feet from dental chair 30 minutes prior to ultrasonic scaling †† during ultrasonic scaling			

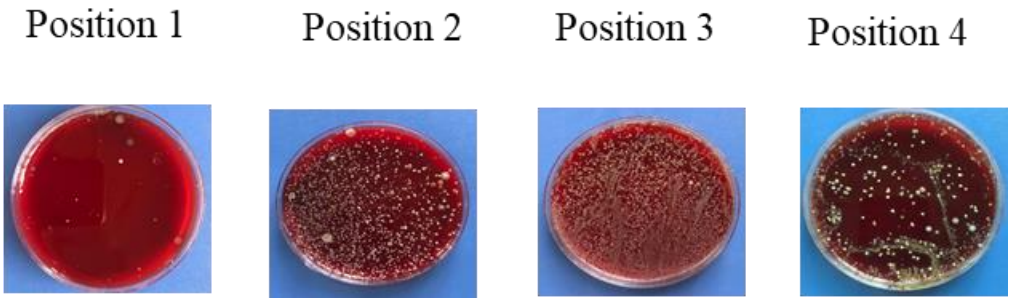

Fig.1: A Schematic representation of microbial colonies in blood agar plates placed at various locations. Note the minimum no of microbial colonies in position 1, while maximum is in position 3 followed by position 2 &4.

IV. DISCUSSION

Aerosols produced during the various dental procedures are potential to spread infection to dental personnel and other individuals in the dental operatory room. This has long been considered as one of the main concerns in dentistry. Greatest amount of contaminated aerosol is observed within 2 feet of the patient, where the dental health professional is usually positioned[11]. This observation reinforces the importance of personal protective barriers like eye shield and face mask, head cap, gloves and gown.

Maximum aerosol production is reported during ultrasonic scaling compared to that of the other dental treatment procedures [6]. Though the complete elimination of the risk posed by dental aerosol is difficult, but may be minimise with the use of a high-volume evacuator, high-efficiency particulate air room filters and ultraviolet treatment of ventilation system during ultrasonic scaling as recommended by ADA[2].

Non-selective culture medium (Blood agar) was used to collect the aerosols produced during ultrasonic scaling. This is a valid medium for culturing the airborne bacteria[16]. On settle down in the blood agar culture medium, bacteria grow and multiply to form clusters of colonies. In this study, these microbial colonies were counted in the agar plates to evaluate the microbial load around the working area during scaling. Three plates were kept at different positions, namely chest of the patients (position 2), operator (position 3) and assistant (position 4) during the scaling procedure to collect the aerosols. Highest number of microbial colonies was observed in the operator's chest area (position 3), which was followed by the chest of the patient (position 2) and assistant (position 4). This indicates that the operator is more prone to aerosol contamination than that of the patients. This finding supports the observation made by Koduganti *et al*., (2014) [14]. However, a large number studies observed a greater number of microbial colonies in the agar plates placed over the patient's chest than that of the operator, explaining the fact larger salivary droplets generated during dental procedures settle rapidly from the air and would heavily contaminate the agar plates on a patient's chest [12, 13]. However, maximum concentration of the microorganisms was observed in aerosols within 2 feet of the patient [11], where the dental health professionals are usually positioned. Again, it is reported that number of microbial colonies decreases with increase in distances from the operating area[2]. It explains the reason of counting the lowest number of microbial colonies in the agar plates placed on the assistant's chest than that of the plates placed over the chest of the operator and patients in our

study. Bentley *et al*., (1994) suggested that distribution of bacterially-contaminated aerosols and splatter is extremely variable and may influenced by type of therapeutic procedure, use of high-volume evacuation, position of the subject in the dental chair, position of the tooth in the mouth that affects the position of the operator relative to the subject, levels of the microorganisms in the subject's mouth, etc.[10],. The reason of highest number of colonies on the operator's chest in this study may be related to the height of the operator that influences the position of the operator. Due to the less height of the operator (DK), the agar plates placed on her chest were probably closer to the patient's mouth than that of the distance between the mouth and chest of the patients. Notably, the observation of this study reinforces the significance of personal protective equipments, such as eye and face shields, head cap, mask, gloves, and gowns/white coats as an additional barrier to cross contamination and minimises the risk of team members and the patients.

The limitation of this study should be considered in interpreting these results. Anaerobic bacteria, viruses and organisms requiring specialized media were not cultured and counted in this study. Moreover, the plate count or "fall out" approach used for collection of the bacteria is subjected to a level of inaccuracy, because bacteria exposed to the air may remain viable, or may lose the ability to form colonies and become non-culturable. Thus, counting only aerobic bacteria may underestimate the true extent of bacterial populations in aerosols[17]. Future studies are necessary to investigate the viable pathogenic organisms generated during use of ultrasonic scalers. To evaluate the levels of airborne bacteria remaining in the operatory room after ultrasonic scaling procedure, culture plates would have exposed post therapeutically as well.

V. CONCLUSION

In the light of the study carried out, we may conclude that more microbial colonies are formed on the agar plates placed on the chest area of the operator than that of the plates placed on the chest area of the patients and the assistants. This states the importance of protection not only for the Dental Surgeon and his assistant, but also for the patient itself from the aerosolised microorganisms generated at the time of ultrasonic scaling procedure.

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