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Research Article

Comparative Evaluation of Microbial Flora of Dental Unit Water Lines Supplied with Distilled Water and Ozone Water in a Dental Operatory

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I N F O

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A B S T R A C T

Introduction: Dental unit water lines (DUWLs) contamination can be treated using various methods. Ozone is a potent bactericide used in the medical industry. The purpose of this study was to find out if there were any beneficial effects of ozone water in a DUWL.

Aims: Comparison of microflora, and colony forming units, when distilled/ ozone water was used in DUWL and to evaluate if ozone cleanses waterlines

Method: The study was carried out in the Department of Prosthodontics & Implantology, SRM Kattankulathur Dental College & Hospital, Tamil Nadu. Twelve dental chairs were split into two groups. Group A and Group B samples utilised distilled water and ozone water respectively in booster bottles. Three samples were collected per dental unit (airotor/ booster bottle/ air/ water syringe) at the beginning and end of the day. Samples were cultured on Nutrient agar and agar-agar to check the growth of microbes and evaluate the count of colony-forming units (CFUs). The data were subjected to ANOVA, Tukey's HSD Multiple Comparison test and independent t tests.

Results: The ozone group (Group B) had no significant reduction in CFUs at the beginning or end of the day, when compared to the distilled water group (Group A). Independent t test revealed a statistically significant ($p < 0.05$) decrease in the microbial count in airotor with ozone water ($p = 0.001$), when compared to the booster bottle and air/ water syringe.

Conclusion: Within the limitations of the study, it was concluded that ozone water in DUWLs can be an efficient method to reduce microflora reaching the mouth and hence prove to be beneficial in immunocompromised subjects.

Keywords: Dental Unit Waterlines, Microbial Colony Counts, Biofilm, Disinfection, Ozone, Infection Control

Introduction

Dental operatories are designed around dental units. The dental unit water line (DUWL) consists of a system of small bore plastic tubes connecting the air/ water syringes, ultrasonic scalers, and high-speed air turbine handpieces to dental units to let the water and air circulate through to activate or cool the instruments.[1] For more than 30 years, contamination in the waterlines of dental units has been known.[2] The sources of contamination include biofilm, which are microscopic colonies of multiplying bacteria, fungi, and protozoa.[2,3] Salivary backflow is another cause of DUWL infection. This can ultimately enter the water bottle (booster bottle), with the resultant contamination of DUWLs.[4-6]

The total heterotrophic plate counts (HPCs) in dental unit water should not exceed 500 CFU/mL, as per the US Centres for Disease Control and Prevention (CDC) Guidelines on Infection Control in Dental Healthcare Settings.[7] DUWLs can be treated using a variety of chemical, physical, or chemico-physical methods, including filtration, flushing, reverse osmosis, chlorhexidine, glutaraldehyde, and chlorhexidine dioxide.[8] The purpose of this study was to use ozone water for the treatment of DUWL. Ozone has exceptionally strong oxidation potential. It has a potent bactericidal effect and is often employed in the medical industry.[9,10]

Methodology

The study was experimental in design and was carried out in January 2023 at the Outpatient Department of Prosthodontics and Implantology, SRM Kattankulathur Dental College & Hospital, Tamil Nadu. Twelve dental chairs (units) were selected randomly and labelled Units 1–12. These 12 units were further split into two groups. Group A consisted of six dental units supplied with distilled water (units numbered 1, 3, 5. 7, 9, and 11) in the booster bottles and Group B comprised the remaining six dental units supplied with ozone water (units numbered 2, 4, 6, 8, 10, and 12). During the course of the day, all prosthodontic procedures were carried out on these units. Ozone water used for the study was generated by an ozone generator at a rate of 100 mg/hr (Figure 1). The water thus generated was added to the booster bottles of Group B dental units. Water from the booster bottle was supplied to the airotor handpiece and air/ water syringe lines.

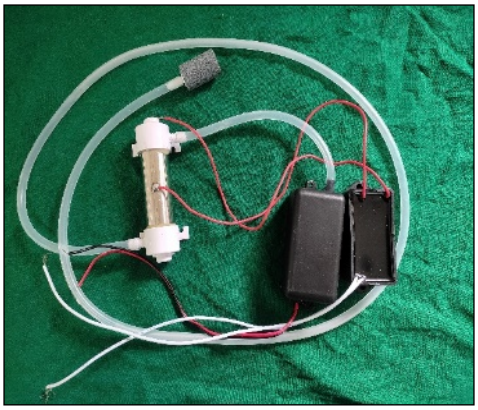

Figure 1.Ozone Generator

Figure 2.Sample Collection from Air/ Water Syringe

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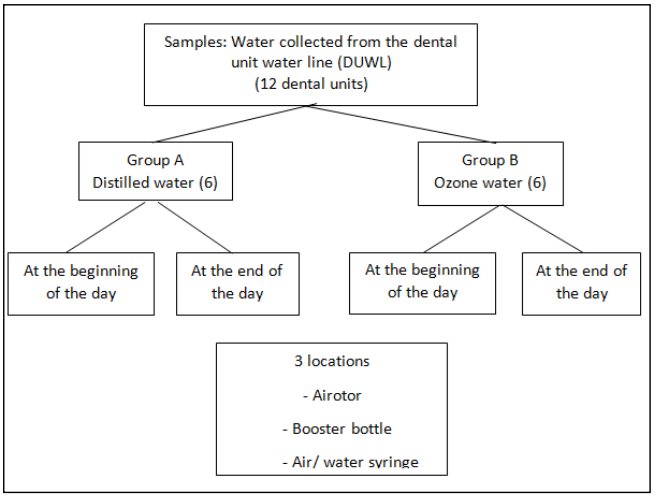

Flowchart 1.Sample Distribution where Group A contained Distilled Water and Group B contained Ozone Water in the Dental Units

Sampling

Early in the morning, all dental treatment water outputs were sterilised with sterillium® (Raman & Weil Pvt Ltd, Mumbai) before samples were drawn.

Samples were collected at the beginning and at the end of the same day, with aseptic precautions. Three samples were collected in three different sterile containers from each dental unit: water from airotor, booster bottle, and air/ water syringe (Figures 2–4). They formed the sub-groups of Groups A and B (Flowchart 1).

A comparison of the microbial flora of DUWLs supplied with distilled water and ozone water was done using microbial culture.

Figure 3.Distilled Water Samples from All the Units

Figure 4.Ozone Water Samples from All the Units

Microbiological Assessment

A single laboratory technician carried out all the procedures in the laboratory. 2.8 g of Nutrient agar (HiMedia, India) along with 1 g of agar-agar was added to 100 ml of distilled water, which was then sterilised by autoclaving at 121 °C and 15 lbs pressure for 15 minutes. After sterilisation, media was poured into sterile Petri plates and was allowed to solidify for 30 minutes. After solidification, a 0.1 ml sample was put into the centre of an agar plate with the help of pipettes and a sterilised glass spreader was used to distribute the sample uniformly throughout the agar surface. At the same time, the Petri dish was rotated carefully. After that, the plate was retained for incubation at 37 °C for 24 hours. Following incubation, the growth of the coliform microbes was analysed and colony-forming units were counted (Figure 5).

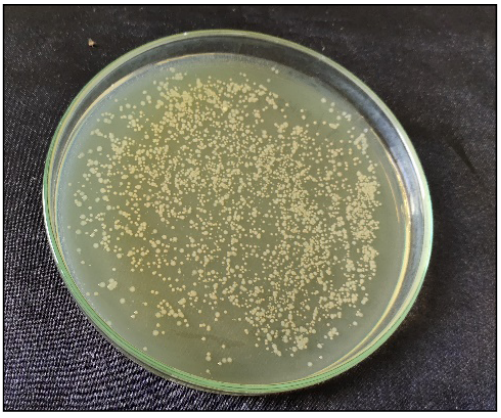

Figure 5.Culture Plate of Distilled Water (Air/ Water Syringe)

Statistical Analysis

The data obtained were subjected to statistical analysis using the SPSS software (Version 28.0.1.1). Analysis of Variance (ANOVA) and Tukey's HSD Post Hoc Multiple comparison tests were applied to Group A and Group B samples individually. Independent sample t test compared

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the samples at the beginning and end of the day and the samples of Groups A and B. The significance was fixed at $p < 0.05$.

Results

Tables 1 and 2 show the comparisons of Group A samples (distilled water) at the beginning of the day, and also between airotor, booster bottle and air/ water syringe.

ANOVA revealed no significant difference in the samples taken from dental units at the beginning of the day in Group A ($p = 0.853$) ($p > 0.05$).

ANOVA revealed no significant difference when the samples were taken from the dental chair at the end of the day in Group A as $p = 0.936$ ($p > 0.05$) (Table 3). There was no statistically significant difference in any of the subgroups with distilled water use at the beginning and end of the day (Table 4).

Tables 5 and 6 show the comparisons of Group B samples (ozone water) at the beginning of the day and also between airotor, booster bottle and air/ water syringe.

ANOVA revealed no significant differences when the samples were taken from the dental chair at the beginning of the day in Group B as $p = 0.619$ ($p > 0.05$). When the data were compared between airotor, booster bottle, and air/ water syringe using multiple comparisons, the p values were found to be not significant.

ANOVA revealed no significant difference when the samples were taken from the dental chair at the end of the day in Group B as $p = 0.644$ ($p > 0.05$) (Table 7). When the data were compared between airotor, booster bottle, and air/ water syringe using multiple comparisons, the p values were found to be not significant (Table 8).

Independent samples t test compared distilled water and ozone water at the end of the day from airotor, booster bottle and air/ water syringe (Table 9). Since the p value was 0.001, it can be inferred that there was a statistically significant difference between the distilled water and ozone water at the end of the day from airotor. Since the p value of the booster bottle was 0.171 and that of the air/ water syringe was 0.066, there was no statistically significant difference between distilled water and ozone water obtained from them at the end of the day.

Table 1.Statistical Analysis using ANOVA for Samples taken at the Beginning of the Day (Group A)

Comparison	Sum of Squares	df	Mean Square	F	Sig.
Between groups	494.333	2	247.167	0.161	0.853
Within groups	23059.667	15	1537.311	-	-
Total	23554.000	17	-	-	-

Table 2.Statistical Comparisons between Different Sites of Samples at the Beginning of the Day (Group A) (Tukey's HSD)

Variable (I)	Variable (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Airotor	Booster bottle	12.83333	22.63707	0.839	-45.9658	71.6325
	Air/ water syringe	6.66667	22.63707	0.953	-52.1325	65.4658
Booster bottle	Airotor	-12.83333	22.63707	0.839	-71.6325	45.9658
	Air/ water syringe	-6.16667	22.63707	0.960	-64.9658	52.6325
Air/ water syringe	Airotor	-6.66667	22.63707	0.953	-65.4658	52.1325
	Booster bottle	6.16667	22.63707	0.960	-52.6325	64.9658

Table 3.Statistical Analysis using ANOVA for Samples taken at the End of the Day (Group A)

Comparison	Sum of Squares	df	Mean Square	F	Sig.
Between groups	259.0	2	129.500	0.067	0.936
Within groups	29079.5	15	1938.633	-	-
Total	29338.5	17	-	-	-

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Table 4.Post Hoc Comparisons between the Different Sites of Samples taken at the End of the Day (Group A) (Tukey's HSD)

Variable (I)	Variable (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Airotor	Booster bottle	9.0	25.42068	0.934	-57.0295	75.0295
	Air/ water syringe	6.5	25.42068	0.965	-59.5295	72.5295
Booster bottle	Airotor	-9.0	25.42068	0.934	-75.0295	57.0295
	Air/ water syringe	-2.5	25.42068	0.995	-68.5295	63.5295
Air/ water syringe	Airotor	-6.5	25.42068	0.965	-72.5295	59.5295
	Booster bottle	2.5	25.42068	0.995	-63.5295	68.5295

Table 5.Statistical Analysis using ANOVA for Samples taken at the Beginning of the Day (Group B)

Comparison	Sum of Squares	df	Mean Square	F	Sig.
Between groups	1981	2	990.500	0.495	0.619
Within groups	30029	15	2001.933	-	-
Total	32010	17	-	-	-

Table 6.Post Hoc Comparisons between Different Sites of Samples taken at the Beginning of the Day (Group B) (Tukey's HSD)

Variable (I)	Variable (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Airotor	Booster bottle	-0.5	25.83237	1.000	-67.5988	66.5988
	Air/ water syringe	22.0	25.83237	0.678	-45.0988	89.0988
Booster bottle	Airotor	0.5	25.83237	1.000	-66.5988	67.5988
	Air/ water syringe	22.5	25.83237	0.666	-44.5988	89.5988
Air/ water syringe	Airotor	-22.0	25.83237	0.678	-89.0988	45.0988
	Booster bottle	-22.5	25.83237	0.666	-89.5988	44.5988

Table 7.Statistical Analysis using ANOVA for Samples taken at the End of the Day (Group B)

Comparison	Sum of Squares	df	Mean Square	F	Sig.
Between groups	1290.333	2	645.167	0.454	0.644
Within groups	21333.667	15	1422.244		
Total	22624.000	17			

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Table 8.Post Hoc Comparisons between the Different Sites of Samples at the End of the Day (Group B) (Tukey's HSD)

Variable (I)	Variable (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Airotor	Booster bottle	-19.83333	21.77341	0.642	-76.3891	36.7225
	Air/ water syringe	-15.16667	21.77341	0.769	-71.7225	41.3891
Booster bottle	Airotor	19.83333	21.77341	0.642	-36.7225	76.3891
	Air/ water syringe	4.66667	21.77341	0.975	-51.8891	61.2225
Air/ water syringe	Airotor	15.16667	21.77341	0.769	-41.3891	71.7225
	Booster bottle	-4.66667	21.77341	0.975	-61.2225	51.8891

Table 9.Independent Samples t Test for Equality of Means

Sample Source	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower Bound	Upper Bound
Airotor	-4.535	10	0.001	-71.66667	15.80225	-106.87627	-36.45706
Booster bottle	-1.477	10	0.171	-42.83333	29.00852	-107.46835	21.80169
Air/ water syringe	-2.060	10	0.066	-50.00000	24.27413	-104.08613	4.08613

Discussion

One of the pillars of excellent clinical practice encompasses efficient prevention of contamination. The prevention of infection in dentistry endeavours to restrict or minimise the microorganisms that are exposed to the patients and dental associates in a dental operatory. Opportunistic infectious agents are often transmitted by patients and practitioners. The possibility of getting infected from dental care is an issue of public concern, particularly due to the fact that this type of care is quite prevalent in the general population and an increasing number of people with immune or medical conditions (such as those with AIDS, cystic fibrosis, chemotherapy patients, or those who have immunosuppressive treatments for organ transplants) are getting regular dental care.[11] However, an additional source might come from the atmosphere, which could be air or water.[12] Nobody would anticipate receiving water that is less than drinking-quality standards in their mouths, despite, being shown in various research that severely contaminated fluid is present in untreated dental waterlines.[13] To eliminate biofilm from DUWLs, antiseptic therapies such as hydrogen peroxide, chlorhexidine gluconate, povidone-iodine, electrochemically activated water, and Listerine mouthwash have been employed so far.[14–18] The findings by McEntegart and Clark demonstrated the effectiveness of disinfectants in reducing CFU in everyday usage.[19]

Ozone has a potent broad-spectrum antimicrobial property that inhibits the growth of bacteria, fungi, viruses, protozoa, and fungal spores. In the disinfection of drinking water and wastewater, ozone is frequently used. Ozone is a much more effective disinfectant than chlorine and other cleaning agents, killing a much wider variety of microorganisms.

Nevertheless, its anti-protozoal effect is yet to be determined conclusively.[20] Azuma et al. in 2014 stated that ozonated water exerts critical anti-inflammatory effects when used in the mouse model.[21] Pak et al. in 2016 studied to assess the efficacy of ozonation in improving the efficiency of removal of antibiotic-resistant bacteria (ARB), under various pH, suspended solids (SS), and humic acid concentrations.[22] Epelle et al. in 2022, analysed ozone stabilisation as a function of detergent concentrations. Additionally, the effectiveness of an ozonated wash in comparison to a regular wash performed with just water was examined and it was found to lessen the negative environmental impact of the resultant wastewater.[23]

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The current study was conducted to evaluate the impact of ozone water in DUWLs contaminated with microflora; and to compare ozone and distilled water for disinfection of DUWLs. The results revealed that ozone water was effective in reducing the growth of coliform bacteria in airotor ($p = 0.001$; $p < 0.05$). This is an important factor as the microbes getting into the subject's mouth during the prosthodontic procedures can be reduced if ozone water is used as booster water. This is an additional benefit for immunocompromised subjects. If the study findings are further validated in a randomised control study, ozone can be used to limit the reintroduction of bacteria through airotors.

In the current study, the effect of ozone in booster bottles and air/ water syringes was negligible. Further investigations are needed to observe the effect of ozone water on these two subgroups. The probable attributes for variations in the results could be that organisms studied may be genetically resistant to such a disinfection method. There can be other factors that need to be considered such as the concentration of ozone used, which may result in a very small reduction in microflora overall. However, this study is only a preliminary investigation with limitations such as the study being conducted on a single day, smaller sample size etc. Other ways of generating ozone could be studied in future studies. The method used to generate ozone in this study was simple and cost-effective.

Conclusion

Numerous studies have highlighted the need to monitor DUWL quality because of the variety of potentially dangerous organisms found in waterlines to ensure the supply of good quality dental patient treatment water.

Within the limitations of the study and based on the findings, it can be inferred that the use of ozone water in DUWLs could prove to be beneficial.

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Conflict of Interest: None

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