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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF THREE DISINFECTANTS IN DISINFECTION OF DENTAL UNIT WATER LINES. A CLINICAL STUDY

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ABSTRACT

Dental unit waterlines may be heavily contaminated with microorganisms and are a potential source of infection for both practicing staff and immunocompromised patients. Contamination of dental unit water lines can be inhibited with the use of disinfectants.

Objective: The objective of this study was to evaluate and compare the efficacy of commercially available Aloe Vera, 3% sodium hypochlorite (NaOCl), and 5gms sodium dichloroisocynurate (NaDCC) in controlling microbial contamination of dental unit water systems.

Materials and Methods: Fifteen dental units, divided into 3groups, were selected for the study. Water samples were collected from the outlet of air/water syringe and high speed hand piece and base line samples were obtained. 500ml of three disinfectants were added to the reservoir bottle. Their efficacy was assessed by the total viable count (TVC) method, after 40 hours, one week and three weeks post disinfection, and was statistically analyzed using one way Anova and wilcoxon signed rank test and post hoc analysis.

Results: NaDCC was found to be the most effective in reducing the microbial colonies, with 99.9% reduction at 40 hours and 100% reduction in mean CFU/ml at 3-week interval of disinfection as compared to sodium hypochlorite and aloevera which was statistically significant (p=0.005).

Conclusions: Decontamination protocol should be followed in order to minimize the risk of exposure to potential pathogens from dental units. Chemical-based disinfectants such as NaDCC can be considered effective, fast acting and economical for treating microbial contamination in dental unit waterlines.

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INTRODUCTION

The presence of microorganism in the dental unit water line was first described by Dr.G.C.Blake in the year 1963, (Kettering *et al*., 2002) since then research is going on to identify various pathogenic organisms and techniques to reduce these microorganism if not completely eliminate biofilm. "Biofilm" is a community of bacterial cells and other microbes that adhere to surfaces and form a protective slime layer (Coleman *et al*., 2009). Dental unit waterlines are an integral part of dental surgery equipment, supplying water as a coolant for air-turbines and ultrasonic scalers. DUWL are susceptible to biofilm formation because they are made from a variety of plastic materials and the tubing has a narrow bore of $1/8^{th}$ to $1/16^{th}$ an inch that provides a high surface area to volume ratio which favors biofilm formation. The low pressure, low flow rates and frequent periods of stagnation of water in the tubing encourages the bacteria to grow inside the tubing (Sarjeev, 2015). The number of immunocompromised patients visiting the dentists is increasing and the water from contaminated DUWL poses a serious risk of infection. The dental professionals too are at the risk of being infected with opportunistic pathogens by means of cross infection or aerosol formation from water emanating from contaminated DUWLs. There are various means by which the quality of DUWL can be improved, that is source water treatment, independent reservoirs, flushing techniques, use of filters and chemical disinfection. Chemical disinfection is a promising method of controlling the biofilm growth and disinfecting the DUWLs (Zanetti *et al*., 2003). These chemicals remove, inactivate, or prevent formation of biofilm. Chemical treatments are either continuously infused into, or are intermittently added to, the dental unit water. (Walker *et al*., 2003) Sodium hypochlorite, is owed to the nature of its chemical reactivity with microbes. Rather than acting in an inhibitory or toxic fashion in the manner of antibiotics, it quickly reacts with microbial cells to irreversibly denature and destroy many pathogens. Sodium hypochlorite, has also shown to react with a microbe's heat shock proteins, stimulating their role as intra-cellular

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chaperone and causing the bacteria to form into clumps (much like an egg that has been boiled) that will eventually die off (Clasen and Edmondson, 2006). Aloe vera is found to have antimicrobial and antifungal properties. It consists of essential oil of *E. camaldulensis*, characterized by the presence of high concentrations of 1,8-cineole with well-documented antimicrobial activity. Essential oils are capable of affecting biofilm formation. They significantly decrease bacterial adhesion and affect bacterial viability in biofilms. The efficacy of aloe vera liquid as an antibacterial agent is shown to have a wide range of effectiveness against Gram-positive (Gram +ve) and Gramnegative (Gram ve) bacteria (Pareek *et al*., 2013).

Sodium dichloroisocyanurateis a WHO certified product, which is been widely used for portable water sanitation. It is an active antimicrobial agent, effective against a wide range of bacteria, fungi, algae, viruses and other microorganisms similar to NaOCl and is more potent when used in same concentration. Sodium dichloroisocyanurate has a sustained release of chlorine (Clasen and Edmondson, 2006). In search of a practical and economical solution to reduce microbial contamination of DUWLs, the present study was conducted to test the efficacy of these three disinfectants.

Aim's & Objectives

- To evaluate the efficacy of Aloe Vera, Sodium DichloroIsocyanurate (NaDCC), and sodium hypochlorite (NaOCl) in controlling microbial contamination of DUWLs, at 40 hours, one and three-week interval.
- To compare the effectiveness of all the three disinfectant use at the different time intervals in reducing the bacterial contamination of dental unit water.

MATERIALS AND METHODS

Fifteen dental units with a closed circuit water system were selected. Before sample collection, the tubing outlet of hand piece and two-way syringe was disinfected with 70% alcohol to avoid other sources of contamination. Water sample was collected in a container without any contact between the hand piece and the container. A volume of 10–40mL of water was collected in sterile containers from the waterlines (triple-syringe, high-speed hand piece). Base line samples were collected at the start of the study (Saturday prior to the end of days' work)

A 500 ml of disinfectant was used, Method of disinfection preparation was as follows:

- GROUP A:250 ml of commercially available aloevera juice (pathanjali) was mixed with 250 ml of distilled water.
- GROUP B: 3% NaOCl in 1:10 concentration 50 ml of NaOCl was mixed with 500ml of distilled water
- GROUP C: 5gms NaDCC tablet (Medichem India) dissolved in 1 lt of water

The concentration of group B & C were adjusted such that both the groups had the same amount of free available chlorine ie 3000ppm of Cl. Each chair received a 500 ml of respective disinfectant in empty reservoir bottle and the solution was run through the system for 3to5 min. The residual disinfectant in the reservoir bottle was left behind and the unit was turned off. The dental units were not used in the weekend for a period of 38 hours. On Monday prior to the start of days' work the disinfectants in reservoir bottle was discarded. The unit was flushed again with distilled water for 2 min to remove the residual disinfectant from the tubing. This disinfection protocol was continued for 3 weeks and water samples of 10–40mL were collected aseptically from the waterlines to quantify the total viable counts, immediately (40 hours post disinfection) one week and three weeks post disinfection. Samples were stored in a refrigerator and processed at the laboratory within two hours of collection of samples. The total viable count was estimated to assess the microbial contamination in the dental unit water line. Standard WHO protocol for incubation for *a period of 48 hours with MacCon Key agar was followed. Automated colony count was used* to assess the microbial cultures, Quantitative bacteriological analysis enumerates total viable population capable of growing under a given set of conditions. The plate count expresses the number of all colony forming bacteria in 1mL of water. The microbial culture reports obtained were subjected to statistical analysis.

RESULTS

The base line samples, along with the water samples with the 3 disinfectants at different time interval were analysed. The intra group comparison was done using wilcoxon signed rank test and inter group comparison was done using Kruskall Wallis One Way Analysis of Variance. Significance of differences was assumed at P _ 0.05 (5%) Table 1 shows the intra group comparision of 3 disinfectants at 40 hours, one week, three weeks post disinfection. Values of all the groups were compared with the base line samples of their respective groups. Aloevera showed 44.2%, 58.56% & 70.00% bacterial reduction at40 hours, one week and three weeks post disinfection. NaOCl showed 81.8%, 95.59% & 99.35% bacterial reduction40 hours, one week, three weeks post disinfection. NaDCC showed 99.9%, 100% &100% bacterial reduction at 40 hours, one week, three weeks post disinfection. All the three groups showed a statistical significant reduction of microbial loads at all-time intervals. (Graph 1)

Table 2: Inter group analysis for disinfection efficacy of the disinfectants was assessed using Kruskall Wallis One Way ANOVA test. The disinfection efficacy of three disinfectants varied significantly ($p<0.002$) with no disinfectant having similar efficacy as the other in reduction of microbial loads. The disinfection potential of NaDCC, NaOCl & Aloevera was found to be 99.9%, 81.8%&44.2% at 40 hours interval respectively, suggesting maximum disinfection activity of NaDCC occurred within 40 hours post disinfection. (Table 2)

To assess the best disinfectant among the three and its efficacy in reduction of microbial loads each disinfectant was compared with the other two disinfectants using Post Hoc analysis.

Table 3 When comparing NaOCl and Aloevera, NaOCl showed significant reduction ($p>0.009$) of microbial loads at all-time intervals. Comparision of NaDCC and NaOCl showed NaDCC had significant reduction ($p<0.009,0.005,0.005$) of microbial loads at 40 hours, one week, three weeks' time intervals respectively. When comparing NaDCC and Aloevera, NaDCC showed significant reduction ($p<0.009,0.005,0.005$) of microbial loads at40 hours, one week, three weeks' time

Table 1. Intra group analysis using wilcoxon signed rank test

Intragroup comparison of disinfectants at different time interval						
		N	Mean	Std. Deviation	% of reduction	p value
Aloevera	Baseline cfu/ml	5.00	124900.00	27258.94		.01
	40 HOURS cfu/ml	5.00	69800.00	11728.38	44.2%	
	Baseline cfu/ml	5.00	124900.00	27258.94		. 005
	1 Week Disinfection cfu/ml	5.00	51760.00	11844.11	58.56%	
	Baseline cfu/ml	5.00	124900.00	27258.94		.002
	3 Week Disinfection cfu/ml	5.00	37480.00	8840.36	70%	
NaOCl	Baseline cfu/ml	5.00	187000.00	43909.00		.002
	40 HOURS cfu/ml	5.00	33900.00	16119.09	81.8%	
	Baseline cfu/ml	5.00	187000.00	43909.00		.001
	1 Week Disinfection cfu/ml	5.00	8260.00	3848.77	95.59%	
	Baseline cfu/ml	5.00	187000.00	43909.00		.001
	3 Week Disinfection cfu/ml	5.00	1220.00	584.81	99.35%	
NaDCC	Baseline cfu/ml	5.00	136600.00	53439.69		.005
	40 HOURS cfu/ml	5.00	140.00	134.16	99.9%	
	Baseline cfu/ml	5.00	136600.00	53439.69		.005
	1 Week Disinfection cfu/ml	5.00	.00	.00	100%	
	Baseline cfu/ml	5.00	136600.00	53439.69		.005
	3 Week Disinfection cfu/ml	5.00	.00	.00	100%	

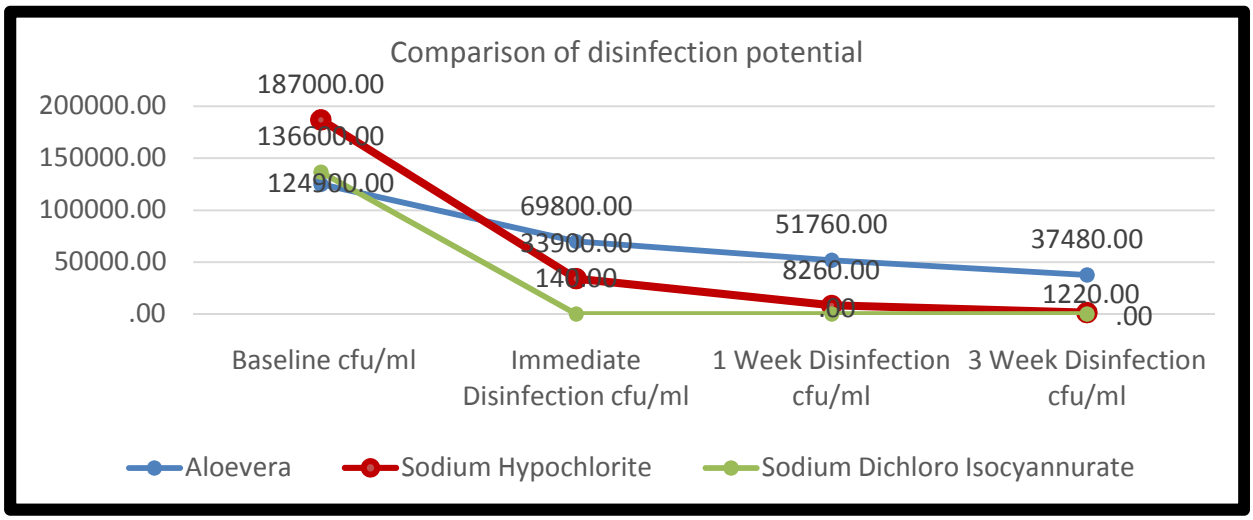
Table 2. Inter group analysis using Kruskall Wallis One Way ANOVA

Time point Groups		N	Mean	Percentage of reduction	Std. Deviation	95% Confidence Interval for Mean		p value
						Lower Bound	Upper Bound	
40 hours Disinfection cfu/ml	Aloevera	5	69800.00	44.2%	11728.38	55237.29	84362.71	.002
	NaOCl	5	33900.00	81.8%	16119.09	13885.51	53914.49	
	NaDCC	5	140.00	99.9%	134.16	-26.59	306.59	
1 Week Disinfection cfu/ml	Aloevera	5	51760.00	58.56%	11844.11	37053.59	66466.41	.002
	NaOCl	5	8260.00	95.59%	3848.77	3481.13	13038.87	
	NaDCC	5	.00	100%	.00	.00	.00	
3 Week Disinfection cfu/ml	Aloevera	5	37480.00	70%	8840.36	26503.24	48456.76	.002
	NaOCl	5	1220.00	99.35%	584.81	493.87	1946.13	
	NaDCC	5	.00	100%	.00	.00	.00	

Table 3. Post Hoc analysis

NaOClVsAloevera			
	40 hours post disinfection cfu/ml	1 Week Disinfection cfu/ml	3 Week Disinfection cfu/ml
Z	-2.611	-2.611	-2.611
p value	.009	.009	.009

NaDCCVsNaOCl			
Z	-2.627	-2.785	-2.785
p value	.009	.005	.005
NaDCCVsAloevera			
Z	-2.627	-2.785	-2.785
p value	.009	.005	.005



Graph 1. Disinfection potential of each group

intervals respectively. The result showed NaDCC was having highest disinfection efficacy which was statistically significant as compared to NaoCl followed by Aloevera at all-time intervals of disinfection.

DISCUSSION

Although contamination of dental unit water systems was identified over 50 years ago, many dentists are still unaware of microbiological contamination or its health risk for dental care providers and patients (Szymańska and Sitkowska, 2013). Due to a combination of negative publicity and an increased scientific knowledge of dental unit waterlines' (DUWL) biofilms and their associated risks, contamination of dental unit waterlines has become a prominent infection-control issue (Pankhurst *et al*., 1998). More than 25 different genera of bacteria have been detected in dental-unit water, along with fungi and protozoa (Tall *et al*., 1995). With the emergence of opportunistic infections in the last few decades, many of these organisms typically recovered from dental unit water systems could prove harmful. These organisms can be amplified in the biofilm to reach infective concentrations, with the potential for inhalation or direct contamination of surgical wounds (Blake Gc, 1963). Research has shown that microbial counts can reach as high as 200,000 CFU/ml within five days after installation of new dental unit waterlines and levels of microbial contamination as high as 106CFU/ml have been documented. (ADA Council On Scientific Affairs (1999)) The cases of death from contaminated DUWL, stresses the importance of monitoring water quality. Mills reported two medical conditions (bacterial endocarditis and a brain abscess) resulted from exposure to contaminated dental treatment water the same strain of gram-negative water bacteria (Moraxella) was isolated from both the patient and the dental unit waterlines. (James and Ketterining, 2002) Recently an 82-year-old Italian woman died due to complications related to Legionnaires' disease, the elderly woman was hospitalized and died 2 days later, and an investigation into the source of the bacterial infection was launched, it was clear that the infection originated from the dental office as patient visited nowhere but the dental office twice over the prior 2-week (Raymond Britto and Muralidharan, 2013). Thus the ADA recommends that there should be no more than 200 CFU/ml of water coming out from the dental unit (Sarjeev, 2015).

According to Watanabe *et al*. (2008) water reservoirs should be cleaned regularly with mechanical and chemical methods to remove the biofilm. In our study the level of bacterial growth from all the water samples collected from the tubing of handpiece and air/water syringe exceeded acceptable levels and were beyond the ADA recommendations. Chlorine has been used as a disinfectant for the treatment of drinking water for more than 100 years. It is by far the most commonly used means of disinfecting water, and its effectiveness as a microbicide has been widely assessed. Until recently, the isocyanurates were used chiefly in the disinfection of water for swimming pools and industrial cooling towers. They are also a common microbial agent in cleaning and sanitizing applications, including baby bottles and contact lens. All of these compounds disinfect water by releasing free available chlorine (FAC) in the form of hypochlorousacid (HOCl)

$$NaCl2\ (NCO)_3 + {}_2H_2O \Leftrightarrow {}_2HOCl + NaH_2(NCO)_3$$

NaDCC dissolution in water.

FAC (chlorine in the +1 oxidation state) is an effective biocide against a wide range of bacteria, fungi, algae, and viruses. Regardless of the original source of the available chlorine, the active microbicidal agent is hypochlorous acid. NaDCC releases only approximately 50% of the chlorine as FAC, the balance remaining as ''reservoir chlorine'' (bound) in the form of chlorinated isocyanurates. When the FAC is used up, the equilibrium is disturbed, immediately releasing further FAC from the ''reservoir'' until the total available is used up. Thus, as shown in the stabilized chlorine in NaDCC acts as a reservoir of HOCl which is rapidly released when the free available chlorine is depleted (Clasen and Edmondson, 2006). In the present study intergroup comparision between NaOCl and alovera showed NaOCl was more potent than aloevera in disinfection which was in contrary to study done by Pareek *et al* where there was no statistical significance between both the groups (Pareek *et al*., 2013). NaOCl can be used for disinfecting spillages of blood containing human immunodeficiency virus or HBV. NaDCC can also be used for this purpose and has the advantages of providing a higher concentration of available chlorine and being less susceptible to inactivation by organic matter (Clasen and Edmondson, (2006). Griffiths *et al*. evaluated the efficacy of several disinfectants (sodium dichloroisocyanurate, sodium hypochlorite, 70% industrial methylated spirits, 2% alkaline glutaraldehyde, 10% succinedialdehyde and formaldehyde mixture, 0.35% peracetic acid and a peroxygen compound at 1% and 3%) against different strains of mycobacteria and showed that disinfectants based on sodium dichloroisocyanurate were more effective after 24 h of optimal incubation conditions (Abreu *et al*., 2013). Four chlorine-based agents commonly used in drinking water treatment and bio film (sodium dichloroisocyanurate, sodium hypochlorite, chloramine-T and chlorine dioxide) were tested for their antibacterial properties for two hours post disinfection. The average log reduction values (LRV) indicated sodium dichloroisocyanurate as the most efficient product in bacterial inactivation followed by sodium hypochlorite, chloramine-T, chlorine dioxide (Anca and Brînduşa, 2014). Above mentioned in-vitro studies on NaOCl and NaDCC showed that NaDCC was superior to NaOCl in disinfection which was in accordance to our study where disinfection levels of 99.9% could be achieved at 40 hours interval. Low cost of 1.5 INR, ease of treatment, compatibility with a wide range of materials, and broad spectrum antimicrobial efficacy, especially against biofilms makes sodium dichloroisocyannurate an effective disinfectant in treatment of DUWL.

Conclusion

- Disinfectants such as NaDCC, may play an important role in reducing microbial counts from DUWLs to more acceptable levels and well within ADA recommendations.
- The use of an economical chemical in the form of NaDCC will prove to be a revolution in disinfecting dental unit water lines.

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