Original Article

Efficiency of a high-speed handpiece with anti-retraction adapter to minimize cross-contamination during the routine dental procedure: A clinical study

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Abstract

Background: This study aimed to detect the efficiency of anti-retraction adapter (ARA) attached to a handpiece (HP).

Materials and Methods: Two types of dental HP with and without the ARA were used in this study. A total of 30 sets of samples were obtained from two groups and were subjected to a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and microbial culture for quantitative analysis of total bacterial and *Legionella* count.

Statistical Analysis Used: The data obtained were tabulated using the Statistical Package for the Social Sciences (SPSS, IBM version 26.0) for statistical analysis.

Results: The water samples were analyzed using PCR, *Legionella*-specific PCR, and culture-based analysis. In Groups 1 and 2, there was no significant difference between bacterial load in the water samples taken from both HP and coupling of the Dental Unit Waterline (DUWL).

Conclusions: The reduction in bacterial load in DUWLs analyzed using quantitative RT-PCR was similar in both experimental groups. Overall, the bacterial load was lower in the group with ARA when compared to the group without ARA but not statistically significant. ARA was not effective in reducing the *Legionella* species load in DUWLs.

Keywords: Anti-retraction adapter; Dental Unit Waterlines; disinfection (MeSH); high-speed dental equipment (MeSH)

INTRODUCTION

The dental chair unit (DCU) is one of the most important equipment in providing dental treatment. It is equipped with intricate narrow bore interconnected flexible tubing called the dental unit waterlines (DUWLs).[1] It is the key component of the DCU that provides water for handpieces (HPs), air/water syringes, and mouth-rinse water outlets.[2] The microbial contamination of DUWLs can occur due to the incoming water of the DCU and by the reverse suction of biological fluids from the oral cavities of patients.[3] As a result of the microbial growth, they adhere to the tube walls and thereby mature into a biofilm which poses health problems to the patient and the dental staff.[2] The contamination is associated with a wide array of microorganisms ranging from bacteria, fungi, viruses, and amoeba.[4] It is a major risk factor for medically compromised or immunologically compromised patients during dental care. An 82-year-old female patient who developed Legionnaire's disease

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after dental health-care appointments was reported in 2012.[5] Various studies in literature have reported that the concentration of bacteria can rise up to 10^4–10^6 colony-forming units (CFUs)/mL.[6]

According to the Centers for disease control and prevention (CDC), all dental units should use systems that treat water to meet drinking water standards (i.e., ≤500 CFU/mL of heterotrophic water bacteria).[7] Dental HPs are one of the critical instruments requiring sterilization for each patient.[8] A dental HP runs at a very high speed which when stops rotating, can retract contaminated fluid from the oral cavity into the HP and also to the DCU which may eventually pave the way for cross-contamination. As routine dental procedures are performed in DCU in patients each day, microbial contamination of DUWL can be a significant cause for cross-infection. The CDC guidelines for infection control in dentistry recommend that dental HPs should be run to discharge water and air for a minimum of 20–30 s after completing the treatment in every patient to reduce the retraction of oral fluids into DUWLs.[7] To overcome the retraction of contaminated water, an anti-retraction adapter (ARA) was developed to be attached to the dental HP.

The ARA works to prevent the backflow of fluids from the oral cavity into DUWLs during the usage of instruments.[9] It is commercially available as an adapter or even inbuilt within an HP. ARA was particularly chosen as it is commercially available and affordable and it can be installed easily onto the HP. The amount of bacterial load contamination could vary with different dental procedures from simple restorative procedure to endodontic treatment. There is only one clinical study that evaluated the efficiency of ARA to reduce Hepatitis-B contamination in high-speed HP.[10] To the best of our knowledge, there is no study in literature that has evaluated the efficiency of HP with ARA (HPWARA) after routine dental procedures. Thus, this study was designed to evaluate the efficacy of ARA with HP to minimize cross-contamination during dental procedures.

Aims and objective

The objective of this study was to assess the efficiency of ARA attached with high-speed HP to prevent contamination in DUWLs after routine dental procedures.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of Meenakshi Academy of Higher Education and Research (MAHER), Chennai (MADC/IEC-III/095/2022) .

Setting of the study

The patients were enrolled from the outpatient unit of the department of conservative dentistry and endodontics. The patients were screened by final-year postgraduate students, not involved in the study. The patients who met the inclusion criteria were recruited for this study after obtaining written informed consent.

Inclusion criteria

Systemically healthy individuals (Category: American Society of Anesthesiologists Class 1) (ASA House of Delegates 2014) aged between 18 and 60 years with maxillary or mandibular teeth diagnosed with G. V. Black Class I, Class II cavity requiring restoration, and teeth requiring endodontic treatment were included.

Exclusion criteria

Patients who are medically compromised and teeth with acute apical abscesses were excluded.

Intervention and clinical procedure

Four final-year postgraduate students well trained in both operative and endodontic procedures performed the dental treatment. Two types of dental HP with and without the ARA were used in this study. The recruited patients were assigned to one of the two groups.

- Group 1: HPWARA-HP with ARA ($n = 15$)
- Group 2: HPWOARA-HP without ARA (HPWOARA) ($n = 15$).

The samples for testing were collected from two areas (HP and coupling without HP [WOHP]), from each dental unit before and after the procedure. Hence, a total of 30 sets of samples were analyzed in each group.

Dental unit water sampling

Dental units that are routinely used for clinical procedures in the department of conservative dentistry and endodontics were chosen. A new DUWL was installed in the two dental units before the start of the study. Dental HP (NSK Pana Air Σ, Nakanishi, Japan) with ARA (NSK, Nakanishi, Japan) was attached to one DCU and an HPWOARA was attached to the second DCU. Routine disinfection protocol was followed before the commencement of the dental procedure. The DUWLs were flushed for 30 s. The HPs and the ARA were sterilized before and after the dental procedure for every patient. Before the procedure, 5 mL water samples were collected from both the HP output water and from the coupling connected to the DUWLs in a sterile container and stored at 4°C for no longer than 24 h before analysis. Protective measures were used during sample collection by the collector. After dental procedures such as restorative care and endodontic treatment, postprocedure water samples were collected similar to the preprocedure sampling. These samples were transported to the laboratory where it was further subjected to microbial investigations.

Bacterial and *Legionella* species count identification using real-time reverse transcriptase-polymerase chain reaction and culture analysis

Bacteria culture and analysis

From the water samples, 10 µL was made as lawn culture on the surface of the sterile brain–heart infusion agar. The plates were incubated at 37°C for 24 h after which the colonies were counted using a digital colony counter. The number of colonies was recorded in the form of CFU/mL.

DNA extraction and real-time reverse transcriptase-polymerase chain reaction assay

From the stored water sample, 1-mL aliquot was thawed and centrifuged at 5000 rpm for 10 min. The sediment was used for DNA extraction with a commercially available kit QIAamp DNA Microbiome Kit (QIAGEN), DNA was eluted in 50 µL of elution buffer (supplied in the kit) and stored at 4°C for up to 12 h before real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis.

Quantitative real-time polymerase chain reaction

The quantitative real-time PCR assay was performed in a 10 µL reaction composed of 1x SYBR Premix (TaKaRa, Shiga, Japan), 1 µL of the extracted genomic DNA, and each of the specific primers. The primers were (314F – CCTACGGGAGGCAGCAG 515R-ATTACCGCGGTGCTGGCA). The assay was performed on the Bio-Rad CFX96 Thermal Cycling System with the following program: 9°C for 3 min, followed by 39 cycles of 95°C for 10 s and 60°C for 25 s annealing temperature for *Legionella*-specific primers (JFP/FAGGGGTTGATAGGTTAAGAG JFP/R CCAACAGCTAGTTGACATCG) and 95°C for 3 min, followed by 39 cycles of 95°C for 10 s and 58°C for 25 s annealing temperature for universal primers. Quantification of total bacteria was performed using the absolute quantification method. Standard dilutions of bacterial DNA were prepared and run alongside the test samples. The standard curve was plotted for the serially diluted samples by employing the corresponding Cycle Threshold (CT) values with which the copy numbers for the test samples were extrapolated and represented as copies/mL. Fluorescence signals were measured every cycle at the end of the extension step. The resulting data were analyzed using CFX Maestro Software, Bio-Rad, California, USA.

Statistical analysis

The data obtained were tabulated using the Statistical Package for the Social Sciences (SPSS, IBM version 26.0, Chicago, IL) for statistical analysis. The independent sample *t*-test was performed for the comparison of microbial load between two experimental groups ($P < 0.05$). The paired sample *t*-test was applied to find out the difference between pre- and postprocedure samples separately in each group. $P < 0.05$ was considered significant.

RESULTS

A total of 30 samples were analyzed for each experimental group with 15 samples taken from HP and 15 samples taken from airotor coupling connected to the DUWLs before and after the dental procedure. Hence, a total of 120 samples were analyzed using culture-based analysis, RT-PCR, and *Legionella* species-specific RT-PCR.

There is no significant difference between both the experimental groups in bacterial load in the water samples taken from both the HP and from the coupling of DUWLs which were tested by both the culture-based method and RT-PCR. Furthermore, the load of *Legionella* species was also not statistically significant [Table 1].

The overall intergroup comparison performed by independent sample *t*-test reveals that there is no statistical difference in bacterial load between the two experimental groups analyzed using RT-PCR and culture-based investigation and also with respect to *Legionella* species-specific RT-PCR before and after dental procedure [Table 2].

DISCUSSION

The literature regarding the efficiency of ARA with high-speed HP is limited. Thus, in this study, the efficiency of ARA used with HP was put to test. The most common method to evaluate the microbiological quality of DUWLs has been the culture-based method. However, these methods underestimate the actual diversity and microbial load of DUWLs.[11] Thus, in our study, we used molecular techniques in addition to the culture-based method. *Legionella* species is one of the opportunistic respiratory pathogens which can survive in varied water conditions.[12] The contamination of DUWLs by *Legionella* species can cause serious health problems to the patients and dental staff. Thus, in this study, the contamination of DUWLs by *Legionella* species was evaluated using RT-PCR. Furthermore, water samples were taken from two different sites (HP output water and from the airotor coupling) in each group as microbes can be retracted into various compartments of HP and lodge in DUWLs following the dental procedure.

In the present study, there was no difference in bacterial load from the water samples taken from HP compared to water samples taken from the coupling connected to DUWLs following dental procedures in both experimental groups. This could possibly indicate that bacterial contamination is similar throughout the water circulating in DUWLs irrespective of the site from which the water sample is taken.

Table 1: Intergroup comparison before and after dental treatment and site of sample collection

	Mean		Std. Deviation		Mean difference	sig. (2 tailed)
(a) Pcr- total bacterial count (in ct value)						
	PRE	POST	PRE	POST		
HPWO-ARA	220492.167	233391.633	133754.755	161161.554	12899.467	0.474
HPWARA	218957.8	229034.2	121831.969	146282.865	-10076.4	0.385
PCR- legionella specific (in CT value)						
HPWO-ARA	18343.955	38449.064	49349.022	116855.806	20105.109	0.132
HPWARA	22862.467	56957.208	63388.981	160524.456	-34094.742	0.305
Microbial culture (in CFU)						
HPWO-ARA	0.347	3.338	1.571	15.081	-2.99	0.291
HPWARA	0.063	2.573	0.156	9.336	-2.51	0.151
(b) Pcr- total bacterial count (in ct value)						
	Mean		Std. Deviation		Mean difference	sig. (2 tailed)
	HP	WOHP	HP	WOHP		
HPWO-ARA	-16206.067	-9592.867	112790.306	82829.698	-6613.2	0.856
HPWARA	9968.267	-30121.067	54347.794	65594.889	40089.333	0.079
PCR- legionella specific (in CT value)						
HPWO-ARA	-15755.838	-24454.38	71175.272	73144.486	8698.542	0.744
HPWARA	27747.319	-95936.803	90986.578	223154.21	123684.122	0.057
Microbial culture (in CFU)						
HPWO-ARA	0.447	-6.427	2.398	21.214	6.874	0.223
HPWARA	-3.534	-1.485	12.089	5.641	-2.049	0.557

*Independent sample *t*-test was used for significance testing. $P \leq 0.05$ is considered significant. HP: Handpiece, WOHP: Without HP, HPWO-ARA: HP without anti-retraction valve, HPWARA: HP with anti-retraction valve, CFU: Colony-forming unit, SD: Standard deviation, PCR: Polymerase chain reaction, RT-PCR: Real-time reverse transcriptase-PCR, CT- Cycle Threshold

Table 2: Overall intergroup comparison of mean bacterial count evaluated using real-time polymerase chain reaction and culture methods and *Legionella* count using real-time reverse transcriptase-polymerase chain reaction

	Mean		SD		Mean difference	Significant (two-tailed)
	HPWO-ARA	HPWARA	HPWO-ARA	HPWARA		
PCR - total bacterial count	−12,899.467	−10,076.4	97,287.66	62,599.678	−2823.067	0.894
PCR - *Legionella* specific	−20,105.109	−34,094.742	71,049.273	178,866.377	13,989.633	0.692
CFU	−2.99	−2.51	15.24	9.327	−0.481	0.883

*Independent sample *t*-test was used for significance testing. $P \leq 0.05$ is considered significant. HPWO-ARA: Handpiece without anti-retraction valve, HPWARA: Handpiece with anti-retraction valve, CFU: Colony-forming unit, PCR: Polymerase chain reaction, SD: Standard deviation

There was an overall reduction of bacterial load from the water samples from both the experimental groups (with and without ARA) before and after dental procedures. However, there was no significant difference between them. This finding proves that ARA could reduce but does not effectively prevent the retraction of fluid to the DUWLs following dental procedures. Our findings are comparable to a previous study by Montebugnoli *et al*. who reported that ARA fails to decrease contamination after dental procedure.[13] Hu *et al*. performed a study to evaluate the risk of hepatitis B virus (HBV) transmission through dental HPs and the effects of an anti-suction device in preventing HBV contamination which concluded that anti-suction devices decrease contamination but do not eliminate it.[10] A study by Fan *et al*. reported that biofilm in DUWLs is related to dental specialty with more abundance of bacterial and fungi communities.[14]

In this study, the contamination of DUWLs by *Legionella* species evaluated using RT-PCR was not different between both the experimental groups before and after the dental treatment. Ditommaso *et al*. performed a study to determine the prevalence of *Legionella* in water from DUWLs and concluded that high rates of *Legionella* contamination in DUWLs.[15] It is proven that conservative dentistry had the highest rate of colonization by *Legionella pneumophila* with air/water syringes demonstrating the highest load of organisms followed by high-speed drills.[16]

Various strategies to reduce the microbial density in DUWLs have been reported in literature which includes both chemical- and nonchemical-based approaches. The chemical methods to disinfect DUWLs are 0.005%–0.02% sodium hypochlorite, 2% glutaraldehyde, 10% iodophors, and 2% quaternary ammonium salts for 3 min. It is highly essential to conduct periodic risk assessment of dental units to identify the risk of contamination. The water supplying the DCU should be within acceptable standards. The standard operating procedure to disinfect DUWLs recommended by the National Health Insurance Administration of Taiwan is flushing

of DUWLs for 3 min before the commencement of the first dental procedure and water flushing of DUWLs for at least 30 s between every patient.[11] The findings from this study reveal that ARA is not effective in preventing contamination of DUWLs. Thus, standard infection control measures along with autoclave sterilization of HPs between patients are essential in dental practice to deliver safe dental care.

Limitations

- The samples were collected after any one of the three procedures, but further testing is required to know the amount of contamination after each and every single procedure
- The samples in our study were collected after any one of the included multiple procedures (deep caries, endodontic procedures, and restorations). However, the results could be different if the samples were collected after a single common procedure (e.g. only endodontic procedures). This needs to be further evaluated.

CONCLUSION

High-speed HPWARA was not effective in reducing the total bacterial load as well as the *Legionella* species load in DUWLs.

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Conflicts of interest

There are no conflicts of interest.

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