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Original Research

Evaluating the shortest and most efficacious decontaminating method for endodontic files in Pediatric dentistry

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ABSTRACT

Aim: To compare and evaluate the effectiveness of various pre-sterilizing techniques used for the sterilization of endodontic files used intermittently in between patients in the dental operatory.

Materials & Methods: The current study involved investigating the effectiveness of 4 methods of pre-sterilizing endodontic files: Control group: no sterilization procedure was performed; Group A- chemical sterilization (with Glutaraldehyde), Group B- Autoclave, Group C- Ultraviolet Chamber and Group D- Ethanol. A total of 50 endodontic files for 10 patients indicated for pulp therapy in primary deciduous molars with 4 root canals each were selected for the study. After access opening, pulp was extirpated by 5 files each per patient.
After access opening, 40 root canals in 10 patients were cleaned and shaped using conventional techniques. Each file was then allotted to each group and sterilized by the above-mentioned methods and assessed for sterility by putting it in Eppendorf tubes containing Brain Heart Infusion (BHI) broth and incubating it at 37^{o}C for 24 hours. The bacterial cultures were then measured for their optical densities using the spectrophotometer.

Results: Maximum decrease in microbial cultures was noted in the Autoclave group followed by the Glutaraldehyde group with a non-significant difference. Ethanol and UV sterilization had non- significant results among them but were significantly different from Autoclave and Glutaraldehyde.

Conclusion: Pre sterilization of files is an important step especially in these times and can be easily achieved by various methods.

Keywords: 70% Isopropyl Alcohol, Cold sterilization, Optical density, Spectrophotometry.

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INTRODUCTION

In modern dentistry, infection control procedures are vital and have a major impact on all the clinical practices.[1] Nowadays, transmission of communicable diseases among patients and dental health care personnel (DHCP) in dental settings is becoming a major concern due to the risk posed by these infectious agents.[2]

Endodontic files are widely used by dentists to clean and shape root canals with the purpose of debriding the root canals chemo-mechanically till the length of the apical foramen.[3] In the case of insufficient infection control while performing endodontic procedures in pediatric dentistry, these instruments can become heavily contaminated with saliva, blood, necrotic tissue and potential pathogens may be transmitted via endodontic instruments which may arise from the root canal systems or from peri-radicular tissues leading to cross infection.[1]

In dental setups, re-sterilization of dental instruments like endodontic files especially in developing countries, for reuse in multiple patients happens on a daily basis.[4] The reuse of endodontic files especially at times like the COVID era, leads to cross infection since instruments directly come in contact with blood, saliva and infected tissues exposing dental practitioners to a high risk of infections like SARS-COV-2 and other communicable diseases. For this main reason, endodontic instruments need to be mandatorily sterilized in order to maintain asepsis to intercept cross-contamination from one person to other.[5] The correct management of sterilization and pre sterilization phases in dental setups play a major role to ensure prevention of cross infection and should be performed with a repeatable, standardizable and documentable method. The complex yet minute architecture of dental instruments like endodontic files makes sterilization difficult. Formulating a pre-sterilization protocol for endodontic files hence requires proper care.[6]

Aseptic procedures are vital for present day dental practice and are repeatedly evolving to meet the high standards of the dental profession. The most common sterilization techniques used in the last thirty years have been autoclaving, glutaraldehyde, and exposure to UV light (240–280 nm). Various authors have considered Autoclaving to be the gold standard for sterilization.[7] Various authors like Sheth et al. 2017, reported Autoclave for 30 mins to be the most efficacious system leading to complete sterilization of endodontic instruments.[8] Germicidal UV radiation is best absorbed by bacterial DNA at a wavelength of 254 nm (240–280 nm) causing crosslinking of nucleic acids within same DNA strand eventually leading to death of micro-organisms.

The current study was designed to evaluate the efficacy of various sterilization methods mentioned for smaller dental instruments like endodontic files which become difficult to clean and to assess which method of sterilization would be best suited to use on a daily basis that would fulfill the need of being efficient, less time consuming, easy to conduct and cost effective.[9] The aim of this study was to compare and evaluate the effectiveness of various sterilization techniques used for the sterilization of endodontic files used intermittently in between patients in pediatric dental practice.

MATERIALS AND METHODS

An experimental type of in-vivo study was conducted during the month of October 2021 at Krishnadevaraya College of Dental Sciences and Hospital, North Bangalore, India where a total of 50 unused K- files of 21mm (Mani k-Files) were removed from the manufacturer's packaging and used. 10 patients within the age range of 5 to 12 years indicated for pulp therapy in primary deciduous molars with 4 root canals were allocated into groups once access opening and confirmation of presence of 4 root canals was done thus following adaptive randomization. Patients who were uncooperative, had poor prognosis of the tooth in question to be treated and those with special needs were excluded from the study. The sample size selected for the study was estimated using the **GPower software v. 3.1.9.4 [(Franz Faul, Universität Kiel, Germany).** Considering the effect size to be measured (f) at 52%, power of the study at 80% and the alpha error at 5%, the sample size needed was 50. Each study group would comprise of 10 samples [10 samples x 5 groups = 50 samples]. The present study was approved by the Krishnadevaraya College of Dental Sciences and Hospital Ethics Committee (KCDSHEC) **[Ethical Approval: KCDS/Ethical Comm/31/2022-23]**. A written informed consent was provided to all the participants of the study. All the procedures in the current study was carried out according to the ethical standards given by the Declaration of Helsinki in 1964, as revised in 2013.

During collection, out of a total of 50 endodontic files, 5 files were used for each patient for pulp therapy. The files were handled using tweezers to remove the files from the packaging by the shaft of the files and contacting the fluted sections of the files was avoided to prevent any microbial contamination. A high speed hand piece with a #18 diamond bur was used to prepare standard endodontic access cavities in the primary molars. ISO size 10 K- files were used to perform the initial instrumentation using circumferential filing to accumulate biological debris and sterile saline was used during the instrumentation to flush out the biological debris. After the root canals were cleaned and shaped using conventional techniques, each file was then cut off from the shaft with the help of a sterile wire cutter each file was then randomly

allocated to each of the below mentioned groups for 15 minutes to investigate the efficacy of the following methods in a short duration to sterilize the endodontic files.

- **CONTROL:** NO STERILISATION PROCEDURE
- **GROUP I:** GLUTARALDEHYDE (2%) for 15 minutes
- **GROUP II:** AUTOCLAVE for 15 minutes
- **GROUP III:** UV CHAMBER for 15 minutes
- **GROUP IV:** ETHANOL for 15 minutes

For each patient, 5 endodontic files were used for instrumentation of the root canals after which 4 files were randomly allocated to the experimental groups and 1 file to the control group. **(Figure 1)**

For **Control group:** no sterilization procedure was performed in this group.
For **Group I:** contaminated files were immersed in a sterile plastic container containing 2% Glutaraldehyde solution for 15 minutes
For **Group II:** the contaminated files were immersed in an endodontic instrument box and subjected to autoclave at 121°C for 15 minutes at a pressure of 15 psi.
For **Group III:** the contaminated files were subjected to UV sterilization for 15 minutes
For **Group IV:** the contaminated files were immersed in a sterile plastic container containing 70% isopropyl alcohol for 15 minutes

After the above-mentioned sterilization procedures, all the files were transferred directly into the Eppendorf tubes containing Brain Heart Infusion (BHI) broth which were incubated at 37º C for 24 hours after which the Eppendorf tubes were vortexed prior to evaluation. The amount of bacterial contamination was evaluated by measuring their optical densities using the spectrophotometer that was made available in the Laboratory of Microbiology, at Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru. **(Figure 2)** The readings were recorded and the results obtained were tabulated and then subjected to statistical analysis. To perform the statistical analysis, a Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released in 2013. Armonk, NY: IBM Corp, was used.

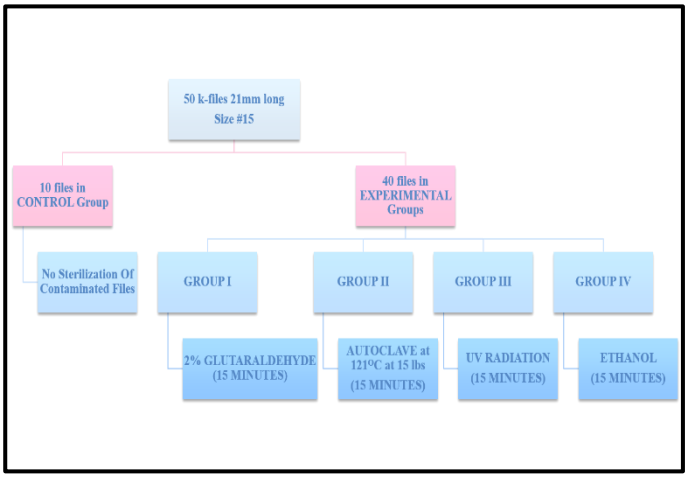

Figure 2

RESULTS

A total of 10 patients with molars having 4 root canals indicated for pulp therapy were selected for the study.
The mean age of the study subjects was 7.5 ± 1.9 ranging between 5-10 years, with 30% of subjects being 8 years of age and remaining age groups at 10-20% distribution. Both males & females were equally distributed [50% each] among the study subjects.

Age & Gender distribution among study subjects			
Variable	Category	n	%
Age	5 yrs.	2	20%
	6 yrs.	2	20%
	8 yrs.	3	30%
	9 yrs.	1	10%
	10 yrs.	2	20%
		Mean	SD
	Mean	7.5	1.9
	Range	5-10	
Sex	Males	5	50%
	Females	5	50%

Table1

Table 2 represents the comparison optical density (OD) values of the microbial load between 5 groups (including the Control Group) where the Kruskal Wallis Test followed by Mann Whitney Post hoc Test was used. The level of significance [P-Value] was set at P<0.05. The test results showed the mean Optical Density of microbial load for Control group was 1.4829 ± 0.4059, Glutaraldehyde group was 0.0796 ± 0.0567, Autoclave group was 0.0259 ± 0.0422, UV group was 0.4653 ± 0.3950 & Ethanol group was 0.3792 ± 0.2598. There was a significant difference observed in the mean optical density among all five groups at P<0.001.

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Comparison of mean OD values between groups using Kruskal Wallis Test						
Groups	N	Mean	SD	Min	Max	P-Value
Control	10	1.4829	0.4059	1.005	2.010	<0.001*
Glutaraldehyde	10	0.0796	0.0567	0.013	0.163	
Autoclave	10	0.0259	0.0422	0.000	0.140	
UV	10	0.4653	0.3950	0.001	1.001	
Ethanol	10	0.3792	0.2598	0.051	0.801	

Table 2: Comparison of mean od values between groups using kruskal wallis test

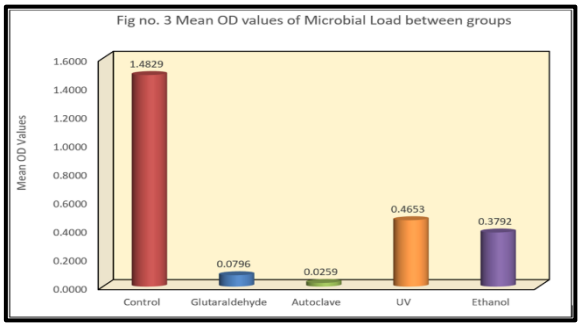


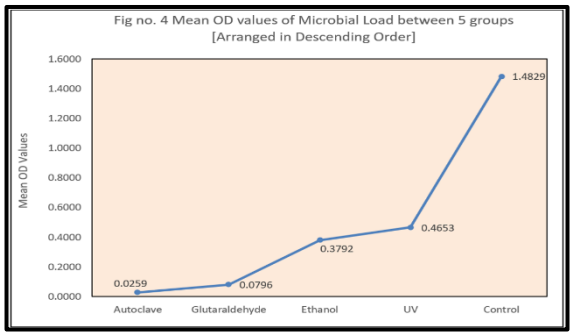
Figure 3 represents the mean optical densities of the microbial load after decontamination with UV method of sterilization showing the most amount of contamination. Results show that **Group I (Glutaraldehyde 2%)** and **Group II (Autoclave)** have optical densities of 0.0796 and 0.0259 respectively whereas Group III and Group IV shows decontamination of only 0.4653 and 0.3792 respectively. This shows Group II to be the best method of sterilization followed by Group I when compared to the other Groups.

Table 3 represents the multiple comparison of mean difference between the groups and revealed that the Autoclave group showed the least optical density of microbial load as compared to Glutaraldehyde at P=0.02, UV group at P=0.006, Ethanol group at P<0.001 & Control group at P<0.001indicating that Autoclave was the most effective group provided maximum sterility. This was then followed next with both Glutaraldehyde which showed significantly lesser OD values as compared to UV & Ethanol groups at P=0.03 & 0.01 respectively and with Control group at P<0.001. This was later observed next with Ethanol & UV groups showing significantly lesser OD values as compared to control group at P<0.001. However, no significant difference was observed in the mean OD values between Ethanol & UV groups [P=0.65].

Multiple comparison of mean difference in OD values b/w groups using Mann Whitney's Post hoc Test					
(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for Diff.		P-Value
			Lower	Upper	
Control	Glutaraldehyde	1.4033	1.0469	1.7597	<0.001*
	Autoclave	1.4570	1.1006	1.8134	<0.001*
	UV	1.0176	0.6612	1.3740	<0.001*
	Ethanol	1.1037	0.7473	1.4601	<0.001*
Glutaraldehyde	Autoclave	0.0537	-0.3027	0.4101	0.02*
	UV	-0.3857	-0.7421	-0.0293	0.03*
	Ethanol	-0.2996	-0.6560	0.0568	0.01*
Autoclave	UV	-0.4394	-0.7958	-0.0830	0.006*
	Ethanol	-0.3533	-0.7097	0.0031	<0.001*
UV	Ethanol	0.0861	-0.2703	0.4425	0.65

Table 3

Figure 4 represents the mean optical densities of the microbial load after decontamination of the endodontic files among the 4 different methods of sterilization. The line graph shows OD values for Autoclave group (0.0259) having the least amount of bacterial load followed by Glutaraldehyde group (0.0796) while the UV method (0.4653) of sterilization showing the highest amount of bacterial load. This could be attributed to the fact that only the microorganisms that are directly struck by UV light beams get destroyed.



DISCUSSION

Steam pressure (Autoclave), dry heat and chemical sterilization are most commonly used to sterilize instruments. As of recent years, the use of UV and even laser as a mode of sterilization has been employed.

Hurtt CA et al. concluded that Autoclave was the best method of sterilization of endodontic files than Glutaraldehyde and salt sterilization. An in-vitro study by Venkata Subramanian R et al, concluded that Autoclave and exposing to laser gives completes sterilization when compared to using Glass bead Sterilizer of Glutaraldehyde.[10]

Another in-vitro study by Raju TB et al.2013, concluded Autoclave to be the best method of sterilization of endodontic files when compared to using Glutaraldehyde, Glass bead sterilizer and CO_2 laser. Yenni M et al. in 2017 concluded that Autoclave revealed complete reduction in the microbial count followed by glass bead sterilizer, Quitanet Plus and

glutaraldehyde.[11] This is in accordance to the present study that Autoclave proved to be the most effective under 15 minutes of endodontic file sterilization in pediatric dentistry.

In the present study, *Autoclave group with OD (*0.0259) followed by *Glutaraldehyde group (*0.0796) showed maximum sterility showing a *non-significant difference.* This is in accordance to a study by Vinay Kumar et al. in 2015 where they concluded that autoclaving and glutaraldehyde (2.4%) revealed complete sterilisation when compared to other methods like using the glass bead sterilizer.[12] Ethanol and UV sterilization had non- significant results among them with 74.4% and 68.6% decontamination respectively but were significantly different from Glutaraldehyde and Autoclave groups.

Although various studies prove Autoclave to be the most efficient method of sterilization is, it tends to rust carbon steel instruments and burs (Rani L et al.2016). Due to the unique nature of dental procedures, which generates large number of aerosols and droplets, the usual standardized protective measures followed by the dental care workers will not suffice for preventing the spread of COVID-19, especially when the patient is symptom-free, unaware about the disease status, or falsifying infection history. Therefore, the containment of the propagation of the virus would be nearly impossible. In today's scenario, this is the major hurdle in establishing a dental home where children are vulnerable for corona virus infections[13]. A systematic review and network meta-analysis by Dioguardi M et al, 2021 concluded that autoclave sterilization protocol must not be repeated more than 5 cycles as it reduces the cutting efficiency of steel and NiTi instruments[14]. Various studies prove that autoclaving may lead to fracture of the rotary instruments due to cyclic fatigue. It has been suggested to use 6% hypochlorite in conjunction which reduces the microbiological load while also improving the fracture resistance[15].

Although UV is used as a surface disinfectant, when it comes to using endodontic instruments, endodontic instruments like files have a *smaller diameter per se* and allows more surface area to come in contact with the light source which could prove UV light to be an be an effective method of sterilizing endodontic files. Sterilization using UV light lamps produces thermal heat which induces the thymine dimers to activate the nucleic acids. The major drawback of the UV method of sterilization is that they are efficacious on only to the surface which comes in direct contact to the light source.[16]

Other acceptable ways of sterilizing endodontic files are the use of chemical sterilization like Ethanol or 2% Glutaraldehyde. Glutaraldehyde has a broad spectrum of bactericidal activity which penetrates into blood and its exudates present on the files due to its low surface tension making it the a frequently used method for cold sterilization *(Kumar et al.2015; Yenni M et al.2017).*[11] Debkant Jena et al. stated that sterilization using glutaraldehyde proved more to destroy the microorganisms from the treated surface in comparison to glass bead sterilization. This is in congruence to the present study that glutaraldehyde is effective enough to sterilize endodontic instruments after autoclave and is a still much better method than using UV or ethanol as a mode of sterilization. Although various studies have also proved that although 2% glutaraldehyde was an acceptable method of sterilizing endodontic instruments, its major drawback is its high toxicity and usually needs a duration of 12 hours to entirely decontaminate the instrument which is a challenge in dental practice.[17]

A spectrophotometer works on the principle of turbidity/optical density determination. As bacteria grow in a broth, the turbidity increases as the number of cells increases allowing less light to be transmitted through it. Hence the spectrophotometer was used in the present study for standardizing and assessing the optical densities of bacterial cultures after using various methods of sterilization.

The present study compared multiple decontamination methods which are easy to use, easily and commonly available and does not require a duration of more than 15 minutes. However, the limitations presented by this study includes its inability to identify the type of microbial contamination involved and use of only traditional endodontic files. Thus, recommendation for future testing should include tests that can identify the source of contamination and also conducting more quantitative and sensitive testing for other endodontic instruments such as Ni-Ti files, broaches, reamers, dental burs, etc. to assess the efficacy of the decontamination methods.

CONCLUSION: Autoclave and Glutaraldehyde can be the most effective, efficient, quick and easily available method for sterilization of endodontic files in between patient procedures in a dental operatory. This can prove beneficial to dental personnel to sterilize endodontic instruments chairside in minutes. Pre sterilization of files is an important step especially in these times and can be easily achieved by various methods in a dental setup.

RECOMMENDATIONS

1. Autoclave can be used as an effective method of sterilization provided it should be noted that the cutting efficiency of endodontic files is drastically affected after 5 cycles of sterilization
2. Using 2% Glutaraldehyde for sterilization of endodontic files can be a faster and more efficient way of achieving complete sterilization
3. UV Radiation can be used as an adjunct to chemical sterilization (2% Glutaraldehyde)

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