Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies ***NLM ID:*** *101716117*

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr Indian Citation Index (ICI) Index Copernicus value = 100

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

Original Research

Evaluation of biological debris on rotary endodontic files subjected to pre-sterilization cleaning and its effect on different sterilization methods: An in vitro study

[1]Malik Sartaj, [2]Omer Hussain Misgar, [3]Amir Rashid Purra, [4]Fayaz Ahmad Ahanger, [5]Riyaz Farooq

[1,2]Registrar, [3]Professor, [4]Associate Professor, [5]Professor and Head, Department of Conservative Dentistry and Endodontics, Government Dental College and Hospital, Srinagar, India

ABSTRACT:

Background: Rotary endodontic files although designed for single use are mostly reused world over increasing the potential for cross contamination. Rotary files get loaded with biological debris during root canal preparation and their cleaning and sterilization is an uphill task owing to their architecture. **Materials & methods:** 50 rotary endodontic files were used to prepare root canals bathed with bacillus stearothermophillus and dived into five groups to evaluate the effect of presterilization cleaning and method of sterilization on the debris burden and sterility of rotary endodontic files before reuse. **Results:** Results showed that mill washing followed by ultrasonic cleaning reduced the debris to negligible levels and steam autoclaving is an effiecient method of sterilization while as glass bead sterilizer can't be relied for sterilization of endodontic files. **Conclusion:** Mill washing followed by Ultrasonic cleaning and autoclaving is an effective cleaning and sterilization method endodontic files with residual biological debris having no significant effect on steam sterilization.

Key words: Sterilization, Endodontic Instruments, Cross Contamination

Received: 11 August, 2022 Accepted: 14 September, 2022

Corresponding author: Omer Hussain Misgar, Registrar, Department of Conservative Dentistry and Endodontics, Government Dental College and Hospital, Srinagar, India

This article may be cited as: Sartaj M, Misgar OH, Purra AR, Ahanger FA, Farooq R. Evaluation of biological debris on rotary endodontic files subjected to pre-sterilization cleaning and its effect on different sterilization methods: An in vitro study. J Adv Med Dent Scie Res 2022;10(10):121-125.

INTRODUCTION

During the past two decades, healthcare-associated infections have become a significant risk to patient safety and acquiring a new infection during episodes of healthcare is a worldwide hazard for both patients and healthcare providers.[1] Many oral and systemic disease agents are easily transmitted by mouth and have long latent period before symptoms appear. Because of the nature of dental care, patients and dental health care personal (DHCP) have the potential for exposure to a variety of microorganisms in dental health care settings. The occupational risk with blood borne pathogens among dental health care workers has been recognized since long. Exposure to infected blood can result in transmission of infection from patient to dental health care person, from dental health care person to patient, and from one patient to another.[2]

Infectious diseases spread by direct contact between individuals, via airborne droplets, or by contact with fomites such as contaminated surfaces or instruments. Contaminated instruments form an important and common mode of transmission.

Although most instruments used in general dentistry also can be used for endodontic therapy, some instruments are designed specifically for endodontic procedures such as rotary files. Most commonly found microorganisms in infected canals are the Gram positive bacteria. In some cases it may be Gram negative and few of the cases yeast. It is currently known that anaerobic microorganisms, especially Gram negative are highly prevalent in root canals of teeth with apical periodontitis.[3] So these files carry the risk of transmitting these bacteria from patient to patient or to health care personal. There is also concern of possible transmission of Prion diseases and endotoxins via contaminated files.[4] The complex,

miniature architecture and irregularities of endodontic files, although not inaccessible makes cleaning and sterilization difficult.[5-7] Whittaker et al found about 1µm thick organic matter on files that had been decontaminated and sterilised, with one third of files contaminant thickness of more than 50µm.[8]

As a result many manufacturers and Department of Health UK 2007 labelled endodontic files as a single use device. But, only in UK 88% of dentists re-process endodontic files after use.[9] Occupational Safety and Health Administration (OSHA) and AAE classify them as reusable sharps and by and large these instruments are still reused after cleaning and sterilization.

Among the various sterilization methods available like hot air oven, autoclave, chemical sterilization by glutaraldehyde, autoclave is a reliable method.[10] But as effective time taken by autoclave for each cycle is more, so glass bead sterilizers were introduced as an alternative for it.[11]

Present study was conducted to evaluate the combined effect of mill washing and ultrasonic cleaning on debris removal and to determine the effect of residual biological debris on sterilization process.

MATERIALS AND METHODS

SAMPLE PREPARATION

Fifty extracted mandibular premolars were decoronated with the help of a diamond disc under water irrigation to obtain a standardized root length of 14 mm measured with digital Vernier calliper. After standardization of the root length, the working lengths of specimens were determined. Specimens were stabilized in sample collection tubes using polyvinyl siloxane material and mounted on customised jig for instrumentation. Initial instrumentation was done with stainless steel hand files upto #20 and 5% NaOCl irrigation.

PREPARATION OF STOCK SOLUTION

Geo bacillus stearothermophillus spore strips containing 10^5 spores were inoculated in Thioglycollate media in 120ml McCartney bottles and incubated at 56ºC in water bath for 48 hours. After incubation broth was sub cultured on Blood Agar plates and incubated overnight at 37ºC for confirmation of growth. The growth of bacillus on Blood Agar plates was confirmed by Gram staining and various biochemical tests. This was taken as stock solution from this 5 ml was transferred to a sterile test tube as a working solution each time procedure was done.

PREPARATION OF ROOT CANALS

Coronal third is enlarged with Sx and S1 rotary Protaper files. 10 microliters of broth from the working solution was injected into root canals of prepared specimens by micropipette and transported along entire length using #15 finger spreader in a pumping action to simulate intra canal infection. Apical preparation is then completed with S1 Protaper files. Twenty randomly selected S1 Protaper endodontic files after instrumentation in teeth inoculated with broth containing B. Sterothermophillus were subjected to cleaning by washer disinfector in a mill wash and then ultrasonic cleaning while other twenty randomly selected files are left without cleaning. These files are divided into two groups each for sterilization in autoclave and glass bead steriliser. So by this we have 4 main groups as ultrasonic cleaning with mill washing by autoclave sterilization, ultrasonic cleaning with mill washing with glass bead steriliser, no cleaning by autoclave sterilization, and no cleaning with glass bead steriliser. The remaining ten files are kept as control.

Group 1: Cleaning with autoclave sterilization (C/A)

Group 2: Cleaning with glass bead sterilization (C/GB)

Group 3: No cleaning with autoclave sterilization (n C/ A)

Group 4: No cleaning with glass bead sterilization (n C/GB)

Group 5: No cleaning no sterilization (control group) (n C/ n S)

Cleaning was accomplished first with washer disinfector for short cycle of 45min in a mill wash unit of vertical rack sterilizer .The recommended detergent containing 5% non ionic surfactant,polycarboxlates,5-15% NTA, enzymes and preservative agents was loaded in bottom chamber of vertical rack sterilization unit. After completion of cycle files are subjected to ultrasonic cleaning in a mill wash for 10 min.

In our study, the endodontic files were sterilized by autoclaving in the same unit, in an instrument box at 121°C for 15 min at a pressure of 15 pounds. Glass bead sterilizer (Confident dental) was used to sterilize remaining files into which whole of the NiTi file were embedded for 10 sec. On completion of the procedure, the files are transferred taking all aseptic precautions into sterile uricol containers. Respective files from each group are collected in separate containers. Fifty percent of the files from each group are subjected to evaluation for debris by SEM and fifty percent for evaluation of sterilization are subjected to microbiological culture.

MICROBIOLOGICAL ANALYSIS OF CONTAMINATION OF FILES

After carrying out the procedure the files were subjected to microbiological evaluation under strict aseptic conditions. Each endodontic file was removed from endodontic motor with a sterile tweezers and then introduced into sterile uricol container separately. Files are then introduced into a test tube containing Thioglycollate media. The test tubes were then incubated at 37ºC for24 hrs. The broth was then sub cultured on blood agar plates. These plates were checked for characteristic growth after 24 hrs of incubation. The growth of Bacillus

stearothermophillus was confirmed by gram staining and various biochemical tests.

ANALYSIS OF DEBRIS IN SCANNING ELECTRON MICROSCOPY

The files were visualized for any debris or contaminant using a scanning electron microscope. The examination was carried out in a clean and dust free environment to try to prevent contamination from dust particles in the air. The debris were visualized at a magnification of 40X. A computer was attached to the microscope in order to save the pictures in the system and also to attain reproducibility of pictures if needed. The whole length of the file was not visible under the microscope under 40X magnification. Therefore the working element of the file, which was 14 mm in length from the silicon stopper, was divided into two equal halves, the tip and the shaft. Each half was photographed and scored for debris.

DEBRIS SCORING

The scale used to measure the amount of debris on the surface of the file was a modification of the scale used by Smith et al.[12]

0 = No debris on the surface of the file.

\+ = 0–5% of the file contaminated with visible debris.

++ = 6–15% of the file contaminated with visible debris.

+++ = 16–25% of the file contaminated with visible debris.

++++ = >25% of the file contaminated with visible debris.

The scoring was blinded by a colleague handing the files to the scorer in a random manner without revealing the identity of the group to which each file belonged. The obtained readings were then statistically analysed.

STATISTICAL ANALYSIS

Statistical software SPSS (version 20.0) and Microsoft Excel were used to carry out the statistical analysis of data. Continuous variables were summarized in the form of means, standard deviations and 95% confidence intervals. Categorical variables were summarized as percentages. Analysis of variance test was employed for intergroup analysis of data and for multiple comparisons;" Least Significant Difference" test was applied. Fisher's exact test was used for comparison of categorical variables. A P value less than 0.05 was considered statistically significant. All P values were two tailed.

RESULTS

Mean debris score and intra group comparison of data for debris removal

The results of the present study demonstrate that ultrasonic cleaning and mill washing although do not completely clean the files but reduce the debris to negligible levels. (Table 1a) There was statistically significant effect of mill washing and ultrasonic cleaning on the debris removal. (Table 1b)

Microbiological evaluation showing percentage of files sterilized among various groups

Results showed that Autoclave is an efficient method of sterilization, sterilizing even the files laden with debris. (Table 2a) Results also demonstrate the inefficiency of glass bead sterilizer, regardless of debris status of file and suggest that glass bead sterilizer can't be relied for sterilization of NiTi endodontic files.

Table 1 (a): Descriptive statistics for percentage of debris

	Mean	SD	95% Confidence Interval for Mean		Min	Max
Group 1	1.0	0.707	0.12	1.88	0	2
Group 2	1.4	0.548	0.72	2.08	1	2
Group 3	21.8	2.387	18.84	24.76	19	25
Group 4	20.6	3.847	15.82	25.38	15	25
Group 5	69.2	15.205	50.32	88.08	50	88

Table 1 (b): Multiple comparison among various groups based on percentage of debris

Group Comparison	Mean Difference	95% Confidence Interval		P-value
1 vs 2	-0.4	-9.78	8.98	0.930
1 vs 3	-20.8	-30.18	-11.42	<0.001*
1 vs 4	-19.6	-28.98	-10.22	<0.001*
1 vs 5	-68.2	-77.58	-58.82	<0.001*
2 vs 3	-20.4	-29.78	-11.02	<0.001*
2 vs 4	-19.2	-28.58	-9.82	<0.001*
2 vs 5	-67.8	-77.18	-58.42	<0.001*
3 vs 4	1.2	-8.18	10.58	0.792
3 vs 5	-47.4	-56.78	-38.02	<0.001*
4 vs 5	-48.6	-57.98	-39.22	<0.001*

*Statistically significant Difference (P-value <0.05)

Table 2 (a): Microbiological evaluation for sterilization among various groups				
Group	Positive		Negative	
	No.	%age	No.	%age
Group 1	0	0%	5	100%
Group 2	3	60%	2	40%
Group 3	1	20%	4	80%
Group 4	4	80%	1	20%
Group 5	5	100%	0	0%

Table 2 (b): Comparison among various groups based on Microbiological evaluation	
Group Comparison	P-value$
Group 1 vs Group 2	0.1667
Group 1 vs Group 3	1.000
Group 1 vs Group 4	0.048*
Group 1 vs Group 5	0.008*
Group 2 vs Group 3	0.524
Group 2 vs Group 4	1.000
Group 2 vs Group 5	0.444
Group 3 vs Group 4	0.206
Group 3 vs Group 5	0.048*
Group 4 vs Group 5	1.000
*Statistically Significant Difference (P-value <0.05), $P-value by Fisher's exact test	

DISCUSSION

Cleaning is an important step in instrument processing because it reduces bioburden and removes material that can harbour endotoxins or prions. Prions are almost impossible to sterilize in dental office and they have affinity for stainless steel. Drying strongly stabilizes the bond of prions to the instruments. So decreasing bioburden before sterilization is imperative. Although, instruments are visibly clean after ultrasonic cleaning but ultrasound alone has been shown to be insufficient for sterilization.[13] We combined the millwashing and ultrasound for presterilization cleaning to reduce the bioburden. The Millwashing (thermo-disinfector) reduces bioburden physically by streams of hot water originating from different directions and thermal inactivation of bacteria on all instruments.

It eliminates the profession-related risk of handling infected instrument. Lets the user go straight to the sterilization and prevents drying of debris on instruments thereby reducing the affinity of prions.

Among the various sterilization methods like hot air oven, autoclave, chemical sterilization by glutaraldehyde which are available, autoclave reliable method.[10,14] But Rapisarda E et al and Neal et al determined that repeated autoclave cycles adversely affect the cutting ability of the files whereas dry heat and glass bead sterlization has no effect.[15] Peter Paul et al evaluate the effectiveness of the glass-bead sterilizer for sterilizing ophthalmic instruments and found it as effective as autoclave.[16] But many studies like by Craig A. Hurtt and Louis E. Rossman have highlighted the inefficiency of glass and salt bead sterilizers in sterilizing the endodontic files.[17]

Previous studies for evaluation of debris or sterilization were either conducted on files collected from general practitioners after clinical use or on extracted teeth without standardization.[18,19] We followed the standardization protocol set by DA Van Eldik et al with slight modifications.[20] For evaluation of debris on endodontic files many techniques including blind examination by an investigator, light microscopy,[6,21] stereomicroscope,[18] Energy Dispersive Spectroscopy (EDS),[22] von Gieson`s stain[23] were used. We took Scanning Electron Microscope for our study because it provides detailed surface data of solid samples and it requires minimal preparation of samples. For evaluating effect of debris on sterilization our study is consistent with results obtained Souza MA et al,[9] Mary A Johnson et al[22] and DA Van Eldik et al[20] which show that, despite the presence of dirt and organic matter on the surface of endodontic files, no bacterial growth was detected after the sterilization by autoclave.

Results of our study also demonstrate the inefficiency of glass bead sterilizer to sterilize endodontic files regardless the presence of debris status of file. Here results of our study are consistent with those Craig A. Hurtt and Louis E. Rossman[17] and CDC guidelines. This suggests that glass bead sterilizer can't be relied for sterilization of endodontic files

CONCLUSION

Within the limitations of the current study it can be concluded that:

1. Mill washing followed by Ultrasonic cleaning and autoclaving is an effective cleaning and sterilization protocol for contemporary rotary NiTi endodontic files.
2. Residual biological debris has no significant effect on steam sterilization of rotary NiTi endodontic files.

REFERENCES

1. Debbie Weston; Infection Prevention and Control: Theory and Clinical Practice for Healthcare Professionals. Wiley and Sons, Ltd.
2. Infection control in Dentistry http://www.dh.gov.uk/asset.
3. Sundqvist G. Ecology of the root canal flora. J. Endod.1992; 18(42):7–30.
4. Sriraman P, Neelakantan P.Asepsis in Operative Dentistry and Endodontics. International Journal of Public Health Science (IJPHS). 2014 Mar; 3 (1):1-6
5. Archie Morrison, Susan Conrod, Dental Burs and Endodontic Files; Are Routine Sterilization Procedures Effective? J Can Dent Ass. Feb 2009; 75(1):39a-39d.
6. Poorva Khullar, Deepak Raisingani. Evaluation of biological debris on reusable endodontic instruments subjected to different cleaning methods prior to sterilization. Int J Infect Control. 2013; 9(2).
7. Parirokh M, Asgary S, Eghbal M. J.An energy dispersive X-ray and SEM study of debris remaining on endodontic instruments after ultrasonic cleaning and autoclave sterilization.Aust Endod J . 2005 Aug;31(2):53-58.
8. Whittaker AG, Grahama EM, Baxtera RL, Jonesa AC, Richardsona PR, Meekb G et al. Plasma cleaning of dental instruments. Journal of Hospital Infection.2004;56(1):37–41.
9. Souza MA, Menin MLF, Montagner F, Cecchin D, Farina AP. SEM and microbiological analysis of dirt of endodontic files after clinical use and its influence on sterilization process. Dental Press Endod. 2011;1:82-6.
10. Venkatasubramanian R, Jayanthi, Das U M, Bhatnagar S. Comparison of the effectiveness of sterilizing endodontic files by 4 different methods: An in vitro study. J Indian Soc Pedod Prev Dent .2010; 28: 2-5.
11. Raju TBVG, Garapati S. Sterilizing Endodontic Files by four different sterilization methods to prevent cross-infection - An In-vitro Study. J Int Oral Health. Dec 2013; 5(6): 108–112.
12. Smith A, Dicksony M, Aitkenz J, Bagg J. Contaminated dental instruments. J Hosp Inf.2002; 51: 233-235.
13. Aslam A, Panuganti V, Nanjundasetty JK, Halappa M, Krishna VH. Knowledge and attitude of endodontic postgraduate students toward sterilization of endodontic files: A cross-sectional study. Saudi Endod J. 2014 Jan-Apr; 4(1):18-22.
14. Boyd KS, Sonntag KD, Crawford JJ. Efficacy of sterilization of endodontic files after autoclaving in a synthetic sponge. Int Endod J. 1994; 27: 330-333.
15. Rapisarda E, Bonaccorso A, Tripi TR, Condorelli GG. Effect of sterilization on the cutting efficiency of rotary nickel-titanium endodontic files.Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999 Sep; 88(3):343-7.
16. Peter Paul T. Ronque, Lee G. Verzosa, Effectiveness of the glass-bead sterilizer in ophthalmic instruments. Philipp J Ophthalmol. 2007; 32(1): 25-27.
17. Craig A. Hurtt and Louis E. Rossman. The Sterilization of Endodontic Hand Files. J.Endod. 1996 Jun; 22(6): 321-322.
18. Ziauddin S, Bhandary S, Pramod J, Srinivasan R, Mahesh MC. A comparative evaluation of the effectiveness of different cleaning protocols on removal of biological debris on endodontic instruments - An in vitro study. Endodontology. Dec 2013; 25(2).
19. Linsuwanont P, Parashos P, Messer HH. Cleaning of rotary nickel-titanium endodontic Instruments. Int Endod J. 2004; 37:19-28.
20. Van Eldik DA, Zilm PS. A SEM evaluation of debris removal from endodontic files after cleaning and steam sterilization procedures. Aust Dent J. 2004; 49:(3):128-135.
21. Letters S, Smith AJ, McHugh S, Bagg J.A study of visual and blood contamination on reprocessed endodontic files from general dental practice. Br Dent J. 2005 Oct; 199 (8):522-525.
22. Johnson MA, Primack PD. Cleaning of endodontic files, Part I: The effect of bio burden on the sterilization of endodontic files. J. Endod. Jan 1997; 23(1):32-4.
23. Popovi J, Gaši j, Stojilkovi G, Stefan Da. Effectiveness of different methods of decontamination of endodontic instruments. Serbian Dental J. 2008; 55(2):88-98