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Original Article

Comparative Evaluation of Three Pre-cleaning Protocols in the Elimination of Biologic Debris on Rotary Nickel Titanium Endodontic Instruments Prior to Sterilization

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Abstract

Introduction: In endodontics, re-use of sterilized instruments is a common practice. The presence of biologic debris reduces the efficacy of sterilization by autoclaving. Thus, pre-cleaning of re-usable endodontic instruments before sterilization is essential.

Aim: Comparative evaluation of three pre-cleaning protocols in the elimination of biologic debris on rotary nickel-titanium endodontic instruments before sterilization.

Materials and Methods: Thirty used rotary S1 Protaper Universal files were randomly divided into three groups for pre-cleaning before sterilization. Group A: 2% sodium hypochlorite + manual brushing, Group B: Ultrasonic bath + Distilled water, Group C: Ultrasonic bath + BIB forte, and Group D: Control group where no pre-cleaning protocol was followed. Following pre-cleaning of endodontic instruments, they were immersed in Rhodamine B dye for 24 h and were mounted on a square block. All the instruments were examined for the presence of residual biologic debris under a stereomicroscope at 30× magnification.

Statistical Analysis: Analysis of variance and *post-hoc* Tuckey's test. The level of significance was fixed at $P = 0.05$, and any value ≤0.05 was statistically significant.

Results: There was a statistically significant difference in the mean value of residual biologic debris between all the groups except Group A and Group D. The mean value of Group C was the lowest.

Conclusion: The combined use of ultrasonic energy and a special enzyme BIB Forte removed the biologic debris to the maximum level.

Key words: BIB forte, Biologic debris, Infection control, Pre-cleaning, Stereomicroscope, Ultrasonic cleaning

INTRODUCTION

Infection control is an important part of every health care unit. With the increasing knowledge of various infectious diseases, the awareness regarding various protocols to achieve optimum infection control is also rising. Dentistry is one of the fields where the operator, the supporting staff and also the patients are exposed to many infectious agents, so proper infection control is paramount.[1,2]

Root canal treatment is routinely performed in every dental clinic. The basic goal of endodontic treatment is the complete removal of all pathogenic debris from the infected root canal. This is achieved with the help of various endodontic instruments and irrigation. In this process, the complex structure of endodontic instruments is accumulated with debris consisting of blood, necrotic tissue, dentinal chips, and numerous infection-causing

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microbes. Thus, if the same endodontic instrument is used in other individuals, there is a definite risk of cross-infection.[2] Hence, it is recommended to use the instrument only once (single use) to achieve appropriate infection control. However, the cost of the instrument being a significant factor, re-use of the instrument is very common, indicating the importance of cleaning the instruments before use and re-use.[3]

Sterilization by autoclaving is the key to achieve optimum infection control. The presence of debris on the surface of the instrument hampers the efficacy of sterilization.[4,5] Thus, pre-cleaning of endodontic instruments before subjecting them to autoclaving is important. At present, used methods for pre-cleaning include manual cleaning with brush, sponge, or gauze. Chemicals such as sodium hypochlorite, chlorhexidine, glutaraldehyde, glass bead sterilizer, and enzymatic cleaners are routinely used. These conventional methods are either cumbersome to perform or do not render the instrument completely free of debris.[6-9] Literature has documented the use of ultrasonic energy as a pre–cleaning method before sterilization. Ultrasonic bath releases acoustic energy and cavitation responsible for the removal of debris from the complex structure of the endodontic file.[6] Considering the limitations of traditional methods and acknowledging the recent devices introduced for pre-cleaning, the vision of the present study was to introduce a pre-cleaning protocol before sterilization which is easy to perform and simultaneously causes near-total cleaning of endodontic instruments.

The aim of the study was to assess the efficacy of ultrasonic bath alone and ultrasonic bath combined with an enzyme BIB Forte in the elimination of biologic debris on rotary nickel- titanium endodontic instruments before sterilization.

MATERIALS AND METHODS

Forty clinically used rotary S1 Protaper Universal files (Dentsply Maillefer, Switzerland) were collected in the following manner. The study was approved by the Institutional Ethical Committee. Participants scheduled for root canal treatment of the mandibular molar having three root canals and vital tooth were selected. Written informed consent was taken from the participants and confidentiality regarding the same was maintained.

Routine endodontic treatment was initiated. Vitality of the tooth was ensured by fresh bleeding from the canals after access opening, failure of which led to exclusion of the participant. Following access cavity preparation and working length determination the glide path was established using the No. 10, 15, and 20 k-file (MANI Inc., Japan) Biomechanical preparation was completed with rotary Protaper Universal files (Dentsply Maillefer, Switzerland) up to F2 sequence installed in an Endomotor (Endo-mate TC2, NSK, Japan) with a speed of 300 rpm and 3 N/m torque. The rotary Protaper Universal files (Dentsply Maillefer, Switzerland) were used in brushing motion with a 3 mm amplitude limit along with gentle apical and lateral pressure. Chemical irrigation was performed with 25 mL of 2.5% NaOCl using a 30 gauge needle inserted up to 1 mm short of working length. 3 mL of 17% EDTA (Prime Dental Products Pvt. Ltd., India) was used for smear layer removal followed by a final rinse with saline. During the biomechanical preparation, all the endodontic instruments were kept on the endodontic stand. Root canal treatment was completed of every participant with F2 Gutta-percha cones (Dentsply Sirona, USA) and AH-Plus sealer (Dentsply, DeTrey, and Konstanz, Germany). Broken, deformed rotary S1 Protaper Universal files were excluded from the study.

Thirty clinically used rotary S1 Protaper Universal files were randomly divided into three groups depending on the pre-cleaning protocol before sterilization and remaining 10 served as a control group.

In Group A, 10 rotary S1 Protaper Universal files (Dentsply Maillefer, Switzerland) were immersed in commercially available 2% sodium hypochlorite (Prime Dental Products Pvt. Ltd., India) for 10 min followed by manual cleaning with 20 strokes of nylon brush (Prime Dental Products Pvt. Ltd., India).

In Group B, 10 rotary S1 Protaper Universal files (Dentsply Maillefer, Switzerland) were immersed in ultrasonic bath (CD-4820 Pvt. Ltd., India) containing distilled water for 15 min. The ultrasonic baths worked at a temperature of 65°C at a power of 160 W.

In Group C, 10 rotary S1 Protaper files (Dentsply Maillefer, Switzerland) were immersed in an ultrasonic bath (CD -4820 Pvt. Ltd., India) containing an enzymatic solution BIB forte (Prime Dental Products Pvt. Ltd. India) for 15 min. 50 ml of commercially available BIB forte was diluted in 950 ml of potable water and was introduced in an ultrasonic bath. The ultrasonic bath worked at temperature of 65°C at a power of 160 W.

In Group D, 10 rotary S1 Protaper Universal files (Dentsply Maillefer, Switzerland) served as a control group. Clinically used S1 Protaper files (DENSTPLY) were not subjected to any pre-cleaning protocol.

After cleaning of instruments in each group, the rotary S1 Protaper Universal files (Dentsply Maillefer, Switzerland)

were kept in covered Petri-dish to minimize exposure to any other contamination. Following this, rotary S1 Protaper Universal files (Dentsply Maillefer, Switzerland) were immersed in Rhodamine B dye (Prime Dental Products Pvt. Ltd., India) for 24 h.

A special holder made of rubber with a square shape was prepared [Figure 1]. The rubber holder had an opening in the center to facilitate easy placement of the rotary S1 Protaper Universal files. The sides of the holder were marked as 1, 2, 3, and 4 corresponding to all four sides of the rotary S1 Protaper Universal file. The holder provided a stable platform and proper positioning of rotary S1 Protaper Universal file under stereomicroscope during the examination of residual biologic debris.

All the rotary S1 Protaper Universal files were examined for the presence of residual biologic debris under a stereomicroscope (SZTP; Olympus Optical Co., Tokyo, Japan) at 30× magnification. The files were divided longitudinally into three sections of equal length with a ruler i.e. coronal, middle, and apical third. All four sides of the rotary S1 Protaper Universal files were examined by sequential rotation through 90°. Digital images of rotary S1 Protaper Universal files were captured with a camera (Nikon Coolpix 950, Nikon, Japan) attached to the stereomicroscope (30× magnifications). Figure 2 is the stereomicroscopic picture of residual biologic debris

Figure 1: File mounted on the rubber block

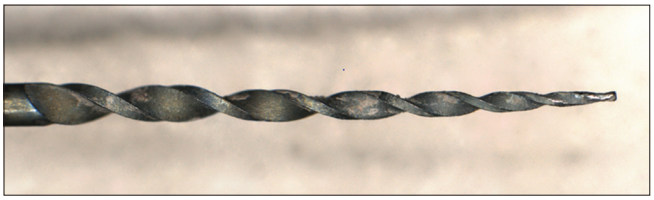

Figure 2: Rotary S1 protaper universal files evaluated for presence of residual biologic debris under stereomicroscope

present on the rotary S1 Protaper Universal file following pre-cleaning. The residual biologic debris on every section was scored as follows:

Score 1- organic film (file covered with a thin unstructured layer), Score 2- slight staining (single separated particles of debris seen scattered over the surface of the file), Score 3- moderate staining (particles of debris seen as a continuous layer over the surface of the file), Score 4- high level of staining (flutes of the file thoroughly covered with debris all over). This criteria of debris classification is same as given by Ziauddin *et al.*[9] Twelve measurements for each instrument were obtained. The minimum value obtained was zero (clean surface with no organic material present) and the maximum was 48 (surfaces of the files were wholly contaminated with debris). All the measurements were added up and the mean value of residual biologic debris was calculated and subjected to statistical analysis.

Statistical Analysis

Analysis of variance and *post-hoc* Tukey's tests were performed using Statistical Package for Social Science (SPSS, version 20.0, USA). The Level of significance was fixed at $P = 0.05$, and any value ≤ 0.05 was considered to be statistically significant.

RESULTS

There was a statistically significant difference in the mean value of residual biologic debris between the pre-cleaning protocols applied in respective groups. The mean value of residual biologic debris for Group A-NaOCl + manual brushing (mean = 2.390) was higher than Group B and C denoting statistically significant difference between Group A, B, and C. $P = 0.05$. However, the mean value of residual biologic debris of Group A was not statistically significant with Group D: control (mean = 2.6420) [Tables 1 and 2].

The mean value of residual biologic debris for Group B- ultrasonic energy (mean = 1.3990) was lower than Group A and D but higher than Group C- ultrasonic energy with BIB Forte (mean = 0.350). There was statistically significant difference between Group B and other groups. $P = 0.05$ [Tables 1 and 2] Lowest mean was obtained in Group C- ultrasonic energy with BIB Forte (mean = 0.350) which was statistically lower than other groups [Tables 1 and 2]. $P = 0.05$ Thus, the combined use of ultrasonic energy with BIB Forte (Group C) was most effective than use of ultrasonic energy alone (Group B).

The mean value of Group D-control (mean = 2.6420) was statistically higher when compared with Group B

Table 1: Comparison of residual biologic debris values in terms of (Mean [SD]) among all the 4 groups using ANOVA test

Group	n	Mean	Std. Deviation	F-value	*P*-value
Group A	10	2.3910	0.42323	77.562	<0.001**
Group B	10	1.3990	0.36452		
Group C	10	0.3750	0.16318		
Group D	10	2.6420	0.46192		
Total	40	1.7017	0.97506		

P<0.05: Significant*, *P*<0.001: Highly significant*, ANOVA: Analysis of variance

Table 2: Tukey's *post-hoc* analysis

Group	Group A	Group B	Group C	Group D
Group A	–	<0.001**	<0.001**	0.442
Group B	<0.001**	–	<0.001**	<0.001**
Group C	<0.001**	<0.001**	–	<0.001**
Group D	0.442	<0.001**	<0.001**	–

(mean = 1.3990) and Group C (mean = 0.350). $P = 0.05$. There was no statistically significant difference between Group A and Group D [Tables 1 and 2].

DISCUSSION

Endodontic files are instruments used during root canal treatment. Procedures carried out in the oral cavity result in the contamination of instruments. The contaminated files can facilitate the transmission of infectious diseases. Hence, it is crucial that efficient infection control practices are implemented before their re-use. Sterilization by autoclaving is considered a fundamental way to achieve infection control. The presence of biologic debris on the surface of endodontic files decreases the efficacy of autoclaving. This highlights the importance of removing debris and bio-burden from the surface of endodontic files. Hence, the pre-cleaning of re-usable files and other instruments is important before presenting them for autoclaving.[1,7]

Soaking instruments in sodium hypochlorite before autoclaving is done since time immemorial. NaOCl dissolves the debris present on the fluted surface of a clinically used instrument.[8] In Group A, the pre-cleaning protocol followed was rotary S1 Protaper universal files were immersed in 2% sodium hypochlorite for 10 min followed by manual cleaning with 20 strokes of nylon brush. This is the easiest and clinically feasible pre-cleaning protocol. Pre-soaking in NaOCl loosens the debris and there is enhanced cleaning by mechanical brushing with a nylon brush.[8]

There were large amounts of residual biologic debris remaining after pre-cleaning protocol followed in this group and this agrees with the study conducted by Parashos *et al*.[6] This may be due to restricted access of bristles to all surfaces of the file blade. The brush is larger than the width of the file flutes. It is unpredictable as to whether the entire circumference of the file is being contacted by the brush.[4] This method is dependent on the human factor and commitment toward making the instrument free of biologic debris leading to the presence of more residual debris.[10]

Rhodamine B dye was used to stain the residual biologic debris. This led to an easy appreciation of debris under a stereomicroscope. In Group B, the clinically used rotary S1 Protaper Universal files were immersed in an ultrasonic bath containing distilled water for 15 min. Ultrasonic cleaning produces high-frequency sound waves which are transferred to the cleaning liquid. This results in the generation and a collapse of a large number of minute bubbles throughout the liquid.[11] Subsequently; these bubbles burst creating water waves that impact the solid surfaces. This effect is known as cavitation which facilitates the cleaning of the instrument.[12]

The pre-cleaning protocol followed in Group B consisted of an Ultrasonic bath and distilled water, while in Group C it was the ultrasonic bath with BIB forte. This was done to assess the efficacy of ultrasound alone and ultrasound combined with an enzymatic solution.

The mean value of residual biologic debris in Group B is lower than Group A and Group D, but the mean value was higher than Group C. This suggests that ultrasound combined with the special enzyme is effective in the removal of debris from clinically used endodontic instruments. This was not in agreement with the study done by Filho *et al*. who concluded that High-Med detergent combined with an ultrasound did not provide greater cleaning efficacy than ultrasonic bath with distilled water indicating that cleaning of endodontic files was due to the ultrasonic energy.[12] The difference in the results can be attributed to different enzymatic solutions and differences in methodology used in these two studies.

The pre-cleaning protocol followed in Group C was rotary S1 Protaper Universal files were immersed in an ultrasonic bath containing enzymatic solution (BIB forte) for 15 min. The lowest values of residual biologic debris were demonstrated in this group. This can be attributed to the synergistic effect of ultrasonic energy and the chemical action of BIB Forte. The ultrasonic bath generates acoustic streaming and cavitation resulting in mechanical flushing of the debris from the complex structure of rotary S1 Protaper Universal files.[13] BIB Forte is active against microbes. It is bactericidal, Tuberculocidal, Mycobactericidal,

fungicidal, and virucidal. It contains special enzymes which are 2.2 g dodecyidipropylenetriamine and 1.7 g of trialkyethoxyammoniumproprionate. It contains surfactant and anticorrosion substance. The chemical action of BIB Forte removes proteins, lipids, and carbohydrates from the instrument surface.[13] Similar findings were obtained by Margana *et al.* who suggested that the enzymatic solution Biosonic due to its chemical action, removes the debris from the surface of the contaminated instrument when used along with ultrasound.[4] Thus, the combined effect of ultrasonic energy and BIB Forte renders the instrument clean prior to sterilization as compared to other pre-cleaning protocols evaluated.

However, accurate quantitative and qualitative analysis of residual biologic debris was not conducted in this study, hence, further research in this regard is needed.

CONCLUSION

The combined use of ultrasound and a special enzyme BIB Forte removed the biologic debris to the maximum level. Further studies with the larger sample using different chemical and mechanical methods for pre-cleaning of instruments before sterilization are warranted. Accurate methods to qualitatively and quantitatively determine the amount of residual biologic debris that are not subjected to human errors are required.

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