“To ascertain effectiveness of pre-sterilization cleaning of endodontic instruments before placement in glass bead sterlizer – An in vitro study”

Shenoi Pratima Ramakrishna¹, Mute Wannala Ramchandra², Makade Chetana Sachin³, Mahajan Anup Kiran⁴*, Singh Harpreet⁵

¹HOD & Professor, ²Professor, ³Associate Professor, ⁴Junior Resident, VSPM Dental College & Research Centre, Nagpur, ⁵Associate Professor, Dept. of Conservative Dentistry & Endodontics, Gian Sagar Dental College & Hospital, Patiala

*Corresponding Author:
Email: anupkmahajan@gmail.com

Abstract
Introduction: In endodontics, reuse of sterilized endodontic instruments is a common practice. During cleaning and shaping of root canal, residual organic and inorganic material accumulates on working sections of endodontic instruments. This debris may act as potential antigens or infectious agents, hence cleaning of instruments play a vital role in effectively sterilizing the instruments.
Aim: Evaluation of pre-sterilization cleaning of endodontic instruments by using 3% H₂O₂ & 2% glutaraldehyde manually & with ultrasonic bath.
Methodology: Fifty, K files (15 No.) were contaminated by preparing canals of extracted human mandibular teeth. Instruments were divided in five groups of 10 instruments each and subjected to different cleaning protocols. The instruments were then immersed in Van-Gieson’s stain and debris was evaluated under stereomicroscope for scoring. The data obtained was statistically analysed using Kruskal-Wallis Test, Wilcoxon Rank Sum Test & Pearson Chi-square Test.
Results: Residual biological debris was observed in 81% of the samples. The sequential cleaning procedure including combined mechanical and chemical removal with 2% glutaraldehyde followed by ultrasonic bath proved to be an effective procedure of removing debris from endodontic instruments. There was a statistically significant difference in the mean values with respect to the cleaning protocol applied (P < 0.001).
Conclusion: Chemical immersion of endodontic instruments in 3% Hydrogen Peroxide & 2% glutaraldehyde showed comparable effectiveness. The ultrasonic method of cleaning instruments in disinfectant was significantly more effective.

Keywords: Biological debris, Endodontic instruments, Infection control, Pre-sterilization.

Introduction
In endodontic practice, microorganisms are the main causative agents for endodontic diseases; hence prevention for transmission of infectious diseases among patients, dentists & its auxiliary staff through proper disinfection & sterilization is of utmost importance.¹,⁶

Endodontic instruments are often contaminated with necrotic & vital tissue, bacteria, dentin chips, blood by-products & other potential irritants which may act as antigens & precipitate spread of infection from one patient to another. This bio burden by forming a protective layer may insulate underlying microorganisms & thus interferes with sterilization.²,⁷,⁹

The geometrical design of endodontic files possesses fluted & twisted sections making mechanical & chemical cleaning considerably difficult. This enhances chances of residual biological debris on the surface of endodontic instruments even after sterilization.¹,⁸

Resterilization of endodontic instruments for reuse on another patient happens regularly in all dental clinics. Owing to their frequent reuse, following a strict sterilization protocol is essential to prevent cross infection.

Literature reveals very few studies investigating effectiveness of cleaning method for endodontic instruments. Segall et al. (1977) suggested chairside cleaning by wiping endodontic instruments with gauze during use. Other researchers like Murgel et al. (1990), Linsuwanont et al. (2004), Parashos et al. (2004), Van Eldik et al. (2004) investigated various cleaning procedures such as mechanical (different kinds of brushes and sponges), chemical (Immersion in various disinfectants, detergents or enzymatic cleaners), ultrasound and a final rinse before sterilization have been used by different authors but none of them mentioned the best cleaning protocol.¹,³

The purpose of our study was to ascertain the effectiveness of pre-sterilization cleaning of endodontic instruments using mechanical, chemical & ultrasonic methods before placement in glass bead sterilizer and to suggest the best cleaning protocol that would be readily incorporated in clinical practice.

Materials and Methods
Fifty, K files (Kendo, Germany, 15 No.) were contaminated by preparing canals of extracted human mandibular teeth & were divided in five groups of 10 instruments each as Group I (Negative control) – New files which were not contaminated.
Group II (Positive control) – Contaminated files without any cleaning protocol...
Group III
a. Manual brushing + 3% H$_2$O$_2$ (Deepti Pharmaceuticals, Nagpur) for 10 min
b. Manual brushing + 2% glutaraldehyde (Raman & Weil Pvt. Ltd., Mumbai) for 10 min

Group IV
a. Manual brushing + 3% H$_2$O$_2$ for 10 min + ultrasonic bath for 5 min
b. Manual brushing + 2% glutaraldehyde for 10 min + ultrasonic bath for 5 min

Group V – Manual brushing + ultrasonic bath for 5 min.

Later the instruments were air-dried, followed by immersion in Van-Gieson’s stain for 3 minutes. They were then rinsed under running distilled water and again air dried. The instruments were then examined for debris at 3 levels apical, middle & coronal using a stereomicroscope (Vardhan, India, Fig.1). A special holder was used in the form of a rubber block (square in cross section) to stabilize the instrument during microscopic examination.¹

According to the criteria specified by Linsuwanont et al. (2004)², the residual debris was categorized as:

a. Stained debris (red or orange aggregates on the surface of the instrument),
b. Organic film (a thin, red unstructured layer covering a part of the instrument),
c. Unstained debris (unstained fine particles) or A clean surface.

Using the amount present as a basis, the residual debris was scored as 0, 1, 2, 3 and 4 as given in Table 1

0 - Clean surface without any debris, 1 - Organic film, 2- Slight staining in the form of single particles of debris scattered on the instrument surface, 3 - Moderate staining, organic particles covering the surface of the instrument as a continuous layer
4 - A high level of staining, with the cutting flutes completely covered with debris. (Table 1)

At each level, the samples were analysed from four sides by sequential rotation through 90°, which resulted in 12 measurements for each sample that covered the entire cutting surface of the instrument. All the measurements were summed & the amount of the biological debris was calculated for each instrument.¹

The scores obtained were analysed using Kruskal-Wallis Test, Wilcoxon Rank Sum Test & Pearson Chi-square Test.

**Results**

Careful examination under the stereomicroscope revealed biological contamination on 81% of the instruments. As shown in Table 2, there was a difference in the quality of cleaning of the instruments that depended on the cleaning protocol applied. This difference was statistically significant($\chi^2=168.5; P < 0.001$) with respect to the amount of residual debris. Microscopic analysis of the new instruments (Group I) that were removed from the packaging of the manufacturer showed that all instruments had a certain amount of unstained metallic debris and only 4% stained particles on their surfaces.

**Table 1: Scoring system for debris on endodontic instruments**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Clean surface without any debris</td>
</tr>
<tr>
<td>1</td>
<td>Organic film</td>
</tr>
<tr>
<td>2</td>
<td>Slight staining in the form of single particles of debris scattered on the instrument surface</td>
</tr>
<tr>
<td>3</td>
<td>Moderate staining, organic particles covering the surface of the instrument as a continuous layer</td>
</tr>
<tr>
<td>4</td>
<td>A high level of staining, with the cutting flutes completely covered with debris</td>
</tr>
</tbody>
</table>

**Table 2: Quantification of debris on endodontic instruments subjected to different cleaning methods**

<table>
<thead>
<tr>
<th>Cleaning Score</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III a</th>
<th>Group III b</th>
<th>Group IV a</th>
<th>Group IV b</th>
<th>Group V</th>
<th>Total</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>-</td>
<td>6(4%)</td>
<td>5(3.3%)</td>
<td>-</td>
<td>2(1.3%)</td>
<td>-</td>
<td>-</td>
<td>13(8.7%)</td>
<td>$\chi^2=168.5$, $P &lt; 0.001$</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>12(8%)</td>
<td>4(2.7%)</td>
<td>2(1.3%)</td>
<td>3(2%)</td>
<td>-</td>
<td>-</td>
<td>21(14%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>8(5.3%)</td>
<td>5(3.3%)</td>
<td>7(4.7%)</td>
<td>3(2%)</td>
<td>2(1.3%)</td>
<td>19(6%)</td>
<td>44(29.3%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6(4%)</td>
<td>4(2.7%)</td>
<td>1(0.7%)</td>
<td>6(4%)</td>
<td>7(4.7%)</td>
<td>9(6%)</td>
<td>11(7.3%)</td>
<td>44(29.3%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>24(16%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4(2.7%)</td>
<td>-</td>
<td>28(18.7%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>30</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>150(100%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Comparison of Group IIIa and IIIb**

<table>
<thead>
<tr>
<th></th>
<th>Group III a</th>
<th>Group III b</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.87±0.51</td>
<td>1.73±0.64</td>
<td>2.33</td>
<td>0.0196,S</td>
</tr>
<tr>
<td>Median</td>
<td>3</td>
<td>1.67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Endodontic instruments are often reused repeatedly during root canal preparation. This possesses great risk of contamination & transmission of infection if cleaning and sterilization protocol is not strictly followed. Considering the need to eliminate all possible links in the chain of contamination, their cleansing, disinfection and sterilisation are well known requirements in dentistry. Letters et al (2005) stated that 75% of the files analysed in their study were contaminated visibly and were accepted sterile to reuse by the practitioners. The data presented in the literature reveals that unused endodontic instruments showed not only metallic, organic particles but also epithelial cells, thus emphasizing the sterilization of even unused instruments. Smith et al. stated that infection is possible because of direct contact of endodontic instruments with the pulpal and periodontal tissues. For effective sterilization it is important to remove residual organic debris, which may prevents direct contact of disinfectant or sterilant or may bind and inactivate its action. Therefore for destruction of viable microorganisms, pre-cleaning of instruments is required prior to their sterilization.

For effective sterilization it is important to remove residual organic debris, which may prevents direct contact of disinfectant or sterilant or may bind and inactivate its action. Therefore for destruction of viable microorganisms, pre-cleaning of instruments is required prior to their sterilization.

Literature available has little information on the optimal removal of biological debris from contaminated instruments. The cleaning procedures that are used include mechanical (different kinds of brushes and sponges) and chemical cleaning (Immersion in sodium hypochlorite, detergents or enzymatic cleaners), ultrasound and a final rinse before sterilization. Hubbard et al (1975) stated that plunging a file into a dry sponge followed by a saline gauze wipe removes 90% of the microorganisms, Segall et al (1977) recommended chair side cleaning of endodontic instruments by using 2” x 2” inch gauze wipes either wet with alcohol or dry. Murgel et al (1990) investigated the effects of a sponge soaked in alcohol and an ultrasonic bath. They found that none of these methods were able to clean the instruments totally and effectively. These manual techniques required considerable amount of time and had risk of reintroducing contamination as were carried out by the human factor.

The objective of our present study was to ascertain & to compare the effectiveness of pre-sterilization cleaning of endodontic instruments by using 3% hydrogen peroxide & 2% glutaraldehyde manually & with ultrasonic bath. In our study individual mechanical, chemical and ultrasonic methods along with their combinations were analysed progressively towards the final protocol.

Van Gieson’s staining method was used to demonstrate organic debris as it is the simplest method of obtaining a differential staining of collagen and other connectivetissue. It’s a mixture of picric acid and acid fuchsin. The cleaning agents used in current study were 2% Glutaraldehyde and 3% hydrogen peroxide. 2% Glutaraldehyde, as it is a strong disinfectant, fixative and kills microorganisms by altering the essential protein compounds. It has been proven to be biocidal in concentrations as low as 2% & has also been reported to be non-corrosive and non-toxic. 3% Hydrogen Peroxide used is reported to produce a transient yet energetic effervescence that displaces debris by bulk flow. The bubbling action of the solution when in contact with tissues physically foams debris out.

The characteristic feature of ultrasonic cleaning is that mechanical energy in the form of high intensity, high frequency sound waves is transferred to the cleaning liquid. This results in generation and collapse of large number of minute bubbles throughout the liquid. This effect is known as “cavitation”. Ultrasonic cleaning in water and disinfectant revealed significantly better cleansing for ultrasonic-disinfectant combination. The present study has shown that the use of ultrasonic is an important step in instrument cleansing and this is in agreement with Rutala et al. If combined with disinfectants, ultrasonic cleansers may have an antimicrobial effect which may reduce residual contamination and enhance safer handling of instruments and more efficient sterilization.

Longer time in ultrasonic may result in the retention of impurities on instrument surfaces and this may also be the case when instruments are not removed from the ultrasonic bath immediately after cleansing but stay in the still detergent solution. Therefore, it is of

Table 4: Comparison of Group IV a and IV b

<table>
<thead>
<tr>
<th></th>
<th>Group IV a</th>
<th>Group IV b</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2±0.52</td>
<td>0.87±0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2</td>
<td>0.67</td>
<td>2.023</td>
<td>0.0431S</td>
</tr>
</tbody>
</table>

Graph 1: Quantification of debris on endodontic instruments subjected to different cleaning methods
great importance to rinse instruments after the treatment to remove the residual contaminated solution.\textsuperscript{3,15,16}

In present study Group I showed that all instruments had a certain amount of unstained metallic debris, but only 4% had stained debris on their surfaces. These results were in accordance with Sonntag & Peters\textsuperscript{1,12}, who found that stained and unstained debris were present on new and unused files after immersion in stain solution. Roth et al\textsuperscript{1,13} found positive bacterial cultures on new endodontic instruments. Therefore pre-sterilization cleaning of unused instruments is also necessary.

When groups III a & III b were compared it was observed that mean residual debris score was 2.87±0.51 in group III a whereas it was 1.73±0.64 in group III b which suggests that 2% glutaraldehyde is more efficient than 3% hydrogen peroxide (P<0.001) [Table 3], whereas when groups IV a & IV b were compared it was observed that mean residual debris score was 2±0.52 and 0.87±0.29 in groups IV a & IV b respectively suggesting 2% glutaraldehyde with ultrasonic bath is more efficient than 3% hydrogen peroxide with ultrasonic bath for pre-sterilization cleaning of endodontic instruments (P<0.001) [Table 4]. These results proved that cleansing protocol is a key factor and emphasize the importance of the mechanical, chemical and ultrasonic decontamination.

The protocol presented in this study relies on chemical agents and equipment rather than on human effort to achieve satisfactory results in cleansing endodontic instruments. Initial manual brushing is easy and can be done quickly. Chemical immersion and ultrasonic cleansing are two very important steps and must be conducted consecutively. Such a protocol is very simple and easy to adopt and apply in both private practice and institutional environment. However qualitative analysis of biological debris is required for which further study is in progress.

**Conclusion**

The common methods used to clean endodontic instruments appear to be generally ineffective for the removal of biological debris. Chemical immersion of endodontic instruments in 3% Hydrogen Peroxide & 2% glutaraldehyde showed comparable effectiveness. The ultrasonic method of biological decontamination of endodontic instruments in disinfectant was significantly (P<0.001) more effective than in water. The complete removal of biological debris from endodontic instruments is feasible by sequential cleaning protocols including combined mechanical and chemical removal with 2% glutaraldehyde followed by placing in an ultrasonic bath.

**Conflicting Interest:** Nil

**Acknowledgement:** Nil

**References**


