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

Shenoi Pratima Ramakrishna[1], Mute Wanmala Ramchandra[2], Makade Chetana Sachin[3], Mahajan AnupKiran[4,*], Harpreet Singh[5]

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

***Corresponding Author:**
Email: anupkmahajan@gmail.com

Abstract

Introduction: In endodontics, reuse of sterilized endodontic instruments is a common practice. During biomechanical preparation of root canal, various organic and inorganic residual debris accumulates on working sections of endodontic instruments. This debris may act as potential antigens or infectious agents, hence cleaning of contaminated endodontic instruments play a vital role in effectively sterilizing the instruments.

Aim: Evaluation of pre-sterilization cleaning of endodontic instruments by using 3% H_2O_2& 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 different cleaning protocols were applied to each group. The selected endodontic 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: 81% of the selected samples showed residual debris. Combination of mechanical and chemical (2% glutaraldehyde) cleaning procedure followed by ultrasonic bath was found to be an effective method of removing debris from endodontic instruments. There was a statistically significant difference in the mean values with respect to the various cleaning protocol applied ($P < 0.001$).

Conclusion: The effect of immersing endodontic instruments in 3% Hydrogen Peroxide&2% glutaraldehyde was comparable. The ultrasonic method was found to be more effective than chemical & mechanical methods.

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.[1,2]

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 micro-organisms & thus interferes with sterilization.[3]

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.[1,4]

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 chair side cleaning by wiping endodontic instruments with gauze during use.[5] Other researchers like Murgel et al. (1990), Linsuwanont et al. (2004), Van Eldik et al. (2004) investigated various cleaning procedures such as mechanical (different types of brushes and sponges), chemical (Embedded 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.[6,7,8]

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_2O_2 (Deepti Pharmaceuticals, Nagpur) for 10 min
a. Manual brushing + 2% glutaraldehyde (Raman & Weil Pvt. Ltd., Mumbai) for 10 min
Group IV – (a) Manual brushing + 3% H_2O_2 for 10 min + ultrasonic bath for 5 min
b. Manual brushing + 2% glutaraldehyde for 10 min + ultrasonic bath for 5min
Group V – Manual brushing + ultrasonic bath for 5 min.

After air drying all the instruments were immersed 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)[7], the residual debris was categorized as:

- Stained debris (red or orange aggregates on the surface of the instrument),
- Organic film (a thin, red unstructured layer covering a part of the instrument),
- 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)

The analysis of contaminated samples was done from four sides at each level by sequential rotation through 90^0.By this each sample has got 12 measurements covering the entire cutting surface of the endodontic 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.

Table 1: Scoring system for debris on endodontic instruments

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

Results

81% of the instruments showed contamination by various debris as seen under stereomicroscope. Various cleaning protocols had significant difference on quality of cleaning of contaminated instruments (Table 2). The amount of residual debris showed statistically significant difference(χ^2= 168.5; P < 0.001). Even the Group I i.e. packed instruments were found contaminated with some amount of unstained metallic debris & 4% stained debris.

Table 2: Assessment of debris on endodontic instruments subjected to various cleaning methods

Cleaning Score	Group I	Group II	Group III a	Group III b	Group IV a	Group IV b	Group V	Total	χ^2
4	-	6(4%)	5(3.3%)	-	2(1.3%)	-	-	13(8.7%)	χ^2= 168.5
3	-	12(8%)	4(2.7%)	2(1.3%)	3(2%)	-	-	21(14%)	P < 0.001
2	-	8(5.3%)	5(3.3%)	7(4.7%)	3(2%)	2(1.3%)	19(6%)	44(29.3%)	
1	6(4%)	4(2.7)	1(0.7%)	6(4%)	7(4.7%)	9(6%)	11(7.3%)	44(29.3%)	
0	24(16%)	-	-	-	-	4(2.7%)	-	28(18.7%)	
Total	30	30	15	15	15	15	30	150(100%)	

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. Cleansing, disinfection and sterilisation are well known requirements in dentistry to avoid chain of contamination.[9] 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.[10] 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.[11]

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.[7]

Very little facts are known about the optimal removal of biological debris from contaminated instruments. Various cleaning procedures are known such as mechanical, chemical, ultrasound and a final rinse before sterilization.[1] 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,[5] 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.[6] 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 which is a mixture of picric acid and acid fuchsin, was preferred to obtain differential staining of collagen and other connectivetissues.[9] The cleaning agents used in current study were 2% Glutaraldehydeand 3% hydrogen peroxide. 2% Glutaraldehyde 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.[12] 3% Hydrogen Peroxide displaces debris by bulk flow by producing energetic effervescence. The bubbling action of the solution when in contact with tissues physically foams debris out.[13]

Ultrasonic cleaning is effective as it produces high intensity, high frequency sound waves which are 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". When Ultrasonic method was used in water and disinfectant, it was observed that ultrasonic-disinfectant combination was significantly better. Popovic et al. showed that instruments cleansing is much better when ultrasonic method was used which is in accordance with the present study[9]. Combination of disinfectants & ultrasonic method gives more efficient sterilization by reducing residual contamination.[9]

Removal of instruments after ultrasonic treatment is most important otherwise retention of impurities on instrument surface may occur.[14,15]

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[16], who found that stained and unstained debris were present on new and unused files after immersion in stain solution. Roth et al[17] 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 element and highlight the importance of the mechanical, chemical and ultrasonic decontamination.

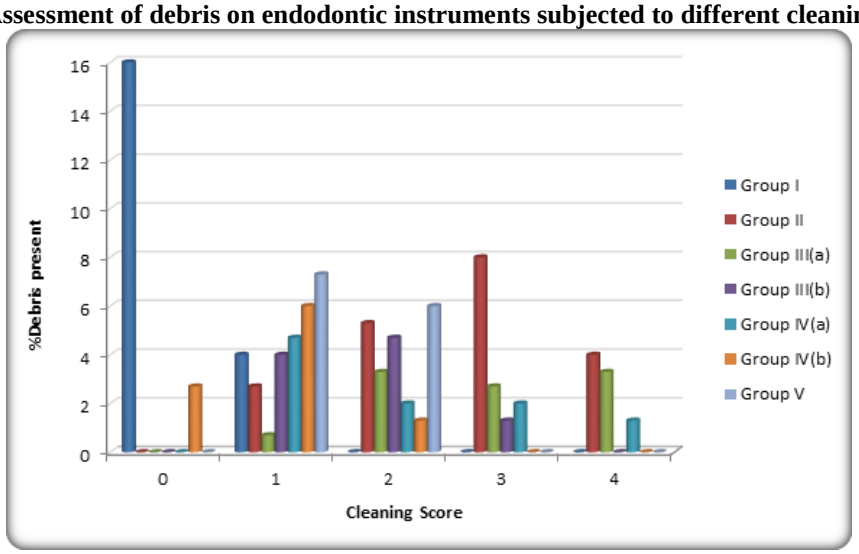
Table 3: Comparison of Group IIIa and IIIb

	Group III a	Group III b	Z value	P value
Mean	2.87±0.51	1.73±0.64	2.33	0.0196,S
Median	3	1.67		

Table 4: Comparison of Group IV a and IV b

	Group IV a	Group IVb	Z value	P value
Mean	2±0.52	0.87± 0.29	2.023	0.0431,S
Median	2	0.67		

Graph 1: Assessment of debris on endodontic instruments subjected to different cleaning methods



The protocol presented in this study depends 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 vital steps and must be conducted accordingly. Such a protocol is very easy to adopt and can administer 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 techniques used to clean endodontic instruments appear to be generally ineffective for the removal of various organic & inorganic 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 consecutive cleaning protocols including combined mechanical and chemical removal with 2% glutaraldehyde followed by placing in an ultrasonic bath.

Conflicting Interest: Nil

Acknowledgement: Nil

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