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Original Research Article

Comparative Evaluation of Apical Extrusion of E Faecalis Using Hand and Rotary Systems

Dr. Mohammed sadique KP[1], Dr. Anshana DM[2], Dr. Ravi SV[3], Dr. Elsy P simon[4], Dr. Sandeep Lal[5], Dr. Mohammed Ashique P[6]

[1,4]Professor, Department of conservative dentistry and endodontics, KMCT dental college, Calicut, Kerala, India
[2]junior resident, Department of conservative dentistry and endodontics, KMCT dental college, Calicut, Kerala, India
[3]Reader, Department of conservative dentistry and endodontics, KMCT dental college, Calicut, Kerala, India
[5,6]Senior lecturer, Department of conservative dentistry and endodontics, KMCT dental college, Calicut, Kerala, India

***Corresponding Author:**
Dr Anshana D M
Email: anshanaajmal3@gmail.com

Abstract: The objective of the study was to evaluate the bacteria extruded apically during root canal preparation using hand and two rotary instrumentation techniques. The method include eighty freshly extracted mandibular premolars were mounted in bacteria collection apparatus. Root canals were contaminated with the pure culture of Enterococcus faecalis (ATCC 29212) and dried at 37 ^{0}c for 24 hour. Then teeth were equally divided into three experimental groups and one control group of 20 each. In group I teeth were instrumented with the hand stainless steel k files in group 2 instrumentation with rotary protaper universal in group 3-instrumentation with rotary protaper next and in group 4-No instrumentation is carried out.Then bacteria extruded were collected ,incubated in brain heart infusion broth for 24 hour at 37 ^{0}c and the colony forming units were counted.The result showed that all hand and rotary instrumentation technique extruded debris.Among all the instrumentation technique hand file extruded more number of debris and protaper next least number of bacteria.

Keywords: apical extrusion, E faecalis, hand file, protaper universal file, protaper next file

INTRODUCTION

The goals of endodontic instrumentation include thorough debridement and disinfection of the root canal system, in addition to creating a suitable shape to achieve a complete three dimensional obturation. In an effort to obtain these goals, debris such as dentinal shavings, necrotic pulp tissue, bacteria and their byproducts or irrigants may be extruded into the periradicular tissue. The extruded material has been referred to as a 'worm' of necrotic debris and has been cited as a major cause of mid-treatment flare-up [1].

Bacteria extruded mainly include gram positive, gram negative bacteria and obligate anaerobes. Microorganism seen in root canal failure cases include Enterococcus faecalis,propionibacterium alactolyticus and propionibacterium propionicum.E faecalis has been identified as a species most commonly recovered from post treatment cases [2].

Apical extrusion of debris has been associated with all types of instruments and instrumentation technique even when the preparation is maintained short of the apical terminus,with some instrumentation technique extrude less material than others.Various factors affect the quantity of apically extruded debris including instrumentation method ,file size and file types [3]. As all instrumentation technique produce some amount of apical extrusion, the choice of technique should also take into consideration how well the apical extrusion of debris can be controlled. Sonic and ultrasonic technique produces less apical extrusion of debris than hand instrumentation. Apical extrusion of debris tends to be greater with hand instrumentation than with technique that use rotary instrumentation because the file may act as pistons that push irrigating solution and debris towards the apex. Conversely rotary instrumentation may move debris along the files which may result in debris being expelled cervically [4]. Reddy and Hicks were the first to compare apical debris extrusion between manual instrumentation and engine driven technique [5].

Nowadays there are lots of rotary instruments. As they differ greatly in their design, type of blade use number of files and kinematics different amount apically extruded debris can be found between the system [6]. Previous study reported that protaper universal rotary file generated large amount debris that

has been related to their aggressive cutting ability recently protaper next system has been introduced [7].

The Protaper next (PTN; Dentsply Tulsa Dental, Tulsa, OK) presents uniqueness with the center of mass and the center of rotation offset design. These files produce a mechanical wave of motion that travels along the active length of the file. This unique design is advantageous in minimizing the engagement between the file and dentin, may also enhance removal of debris out of a canal and improves flexibility of the files [8]. Limited data concerning protaper next files are currently available.

Thus the aim of the study was to compare the apical extrusion of bacteria using hand stainless steel K file, protaper universal and protaper next. The null hypothesis tested was that there is no difference between the amount of apically extruded debris associated with hand and protaper universal and protaper next system.

EXPERIMENTAL SECTION

A total of 80 freshly extracted human single-rooted mandibular premolar teeth with complete root formation were selected. Teeth with one root canal and one apical foramen and an apical diameter corresponding to #10 K-file, were selected. Periapical radiographs were taken to confirm that all the samples had a patent single root canal with single apical foramen. The curvature of the root was determined using Schneider's technique and only teeth with curvatures from 0 to 10 degrees were included to eliminate the complications likely to occur in a severely curved root canal. Teeth with calcification and open apices were excluded. The teeth were cleaned of debris and soft tissue remnants and stored in physiological saline solution until required. Endodontic access cavities were prepared (Endo Access Bur, Dentsply Maillefer, Ballaigues, Switzerland) in a high-speed hand piece and pulpal remnants were extirpated using a broach. The working length was determined by placing a #15 K-file until it was just visible at the apical foramen. From this, 1 mm was subtracted to determine an accurate working length.

Preparation of test apparatus

Two coats of nail varnish applied to the external surface of the root and then the tooth was forced through the rubber stopper of a vial. 27-gauge needle was inserted into the vial through the rubber stopper to equalize the air pressure. The hole was created in nail varnish that covered the apical foramen using 10 K-file. In this way, the standard size foramen and apical patency was achieved. Entire apparatus was sterilized in an autoclave.

Before experiment, the vial was filled with normal saline solution. The same procedure was repeated to all experimental teeth (figure 1).

Fig-1: Test apparatus

Preparation of enterococcus faecalis

A pure culture of E. faecalis (ATCC 29212) was used to contaminate the root canal. Suspension was prepared by adding 1 ml of pure culture of E. faecalis grown in brain–heart infusion broth for 24 hour to fresh brain–heart infusion broth. The McFarland standard number 0.5 was used to evaluate the broth to ensure that number of bacteria was 1.5×10^8 colony forming units (CFU) ml/l. Root canal was completely filled with the E. faecalis suspension. During incubation, canals were hand instrumented with #10 K-file to carry the bacteria down the length of the canal. The contaminated root canal was dried at 37°C for 24 hour.

Samples were equally divided into three experimental groups and one control group of 20 teeth.

Group I – Teeth in this group were instrumented with the hand stainless steel k files

Apical preparation starting at the apical constriction till #25 K-file. preparation of remainder of the canal, gradually stepping back while increasing the instrument #30 to #50 done. Frequent recapitulation using #10, #15, #20, and #25 K-files as larger size files are used for apical preparation.

Group 2-Instrumentation with rotary protaper universal. After achieving a straight-line access; a smooth glide path was achieved with, #10 or #15 K-file used till two-third the working length. Sx files were than used sequentially until resistance was encountered(4–5 mm from working length) followed by S1 and S2 to working length for shaping of coronal two thirds of the canal. Using F1, F2, files sequentially to the working length finishing of apical third was done.

Group 3: Protaper next instruments were used according to the manufacturer's instructions using a gentle in-and-out motion with a torque-controlled

endodontic motor at 300 rpm and a torque of 2.6 Ncm.The root canal orifice was flared using Sx file from the universal ProTaper (Dentsply Tulsa Dental, Tulsa, OK). This was followed by the use of X1 and X2 files. On meeting obstruction the file was removed, the canal was irrigated, recapitulated, and the file was re-introduced into the canal again. The instrumentation was continued till the X1 and X2 both reached the working length.

Group 4 (control group): no instrumentation was carried out during root canal instrumentation, 0.9% normal saline was used as an irrigant after each instrument change. The rubber stopper was placed on the needle and the needle was advanced into the root canal 3 mm short of working length. The apical preparation was done till #30 K-file in all instrumentation techniques.

Subsequently after root canal preparation 0.1 ml of saline was taken from experimental vial in order to count the bacteria and incubated in brain–heart infusion agar at 37°C for 24 hour. Colonies of bacteria were counted using a colony counter (Yarco colony counter) following a classical bacterial counting technique as described by Collins *et al*. The results were given as number of CFU ml/1.

Statistical analysis was done using SPSS software. It was analyzed using One‑way ANOVA and post hoc independent t‑test. The level of statistical significance was set at P value = 0.05. Results were expressed as CFU/ml.

RESULTS

Data regarding the number of bacteria extruded are presented in Table 1. The results indicated that all instrumentation techniques tested caused a measurable apical extrusion of bacteria.

Maximum bacterial extrusion was seen group 1 followed by group 2 (figure 2 and 3). Group 3 showed least apical extrusion of bacteria followed by group 4 (figure 4 and 5).

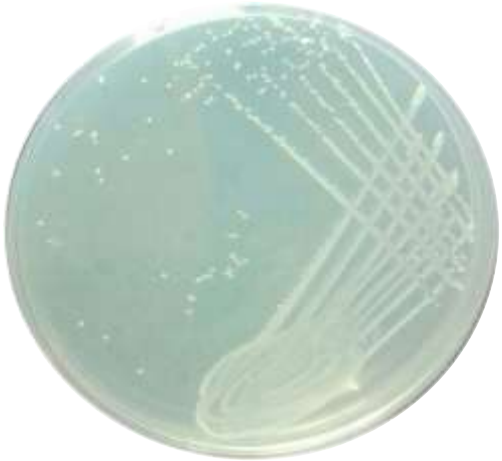

Fig-2: Bacterial extrusion in Group-1

Fig-3: Bacterial extrusion in Group-2

Fig-4: Bacterial extrusion in Group-3

Fig-5: Bacterial extrusion in Group-4

Pairwise comparison among different groups showed P < 0.05 indicating significant differences between the groups.

Table 1: Mean, SD and *P* for four different file systems including the control group

Group	Instrument Type	Mean+/-SD (CFU/Ml)	P Value For Intergroup Comparison		
			Hand	Protaper Universal	Protaper Next
Group 1	**Hand Type**	790±48.475		0.00035	0.00020
Group 2	**Rotary Protaper Unniversal**	640±74.387	0.00035		0.00025
Group 3	**Protaper Next**	475±62.765	0.00020	0.00025	
Group 4	**Control**	0±0	0.00000	0.00000	0.00000

P<0.05 shows a significant difference between groups at α=0.05 level of significance (sig=statistically significant difference). SD=Standard deviation, CFU=Colony forming units.

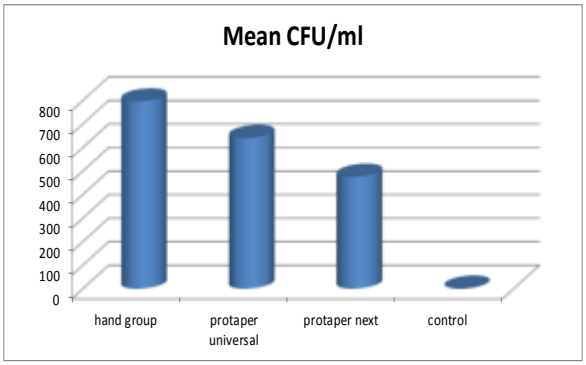

Fig-6: Comparison of Mean CFU/ml in various group

DISCUSSION

Torabinejad *et al* stated that physical and chemical injury of periradicular tissue during cleaning and shaping of root canal system can cause degranulation of mast cell in periradicular tissues. Mast cells discharge vasoactive amines in to the periapical tissues and initiate inflammatory response or aggravate an inflammatory process [8].

Seltzer S and Naidrof *et al* studied the immunological aspect of post-operative flare ups and concluded that antigen originating from the root canal resulting the formation of antigen antibody complex when forced beyond the apical foramen ,which may lead to severe inflammatory response.

The intensity of inflammatory response will depend on the number (quantitative factor) and virulence (qualitative factor) of bacteria. When virulent clonal types of bacterial species are propelled into periapical tissue, even small amount of infected debris can exacerbate the periapical inflammation [9].

The aim of the present study was to assess the extrusion of intracanal bacteria as a result of canal shaping by different instrumentation technique.

Methodology employed in this study was similar to that described by Er *et al*. E faecalis was used as the bacteriological marker as it is implicated in persistent root canal infection and is identified as the species most commonly recovered from root canals of teeth post treatment diseases.it is non-fastidious, easy to grow, facultative anaerobic bacterium and is reported to survive alone without symbiotic support from other bacteria [2].

Several factors affect the amount of intracanal bacteria extruded including the tooth instrumentation technique, instrument type, size and preparation end point and irrigating solution

In the present study only single rooted mandibular premolar were used because application of one kind of tooth can help increase the similarity between specimens. The teeth were carefully selected according to the tooth type canal size, working length and canal curvature. The teeth were radiographed from clinical and proximal aspects to ensure that they had single canal and single orifices [3].

Apical patency was maintained with 10 k file in all cased to achieve standardization of apical diameter [10]. Tinaz *et al* showed that as the diameter of apical patency increased the debris extrusion also increased [10] while Lambrianidis et al paradoxically reported that greater amount of extrusion occurred when the apical constriction remain intact [11]. Two other studies found no correlation between the amount of debris extruded and apical diameter [12].

The apical diameter of master apical instruments in all the groups was standardized at ISO Size 25 to avoid any variations in the amount of extruded bacteria due to the size of apical enlargement. Therefore, apically extruded debris from root canal specimens could be attributed to the design and technique of the respective instrument used in that particular group [13].

Normal saline was used as irrigating solution as it has no antibacterial effect. Hence extrusion and elimination of bacteria depended only on mechanical action of instruments [2].

In this study using engine driven nickel titanium instruments for the canal extruded less debris than k file. In case of engine driven instruments early flaring of the coronal part of the preparation may improve instrument control during preparation of apical third of the canal. The rotary motion tend to direct debris towards the orifice avoiding its compaction in the canal.

In case of K file the reason for more apical extrusion of debris is that the file acting at the apical third act as piston that tend to push the debris through the foramen and less space is available to to flush it out coronally [5].

When comparing the two rotary systems protaper next was found to be better than protaper universal. Both the rotary files tested have different alloy properties and cross sectional design. The PTU instruments have convex triangular cross-section design,non cutting safety tip and flute design that combines multiple tapers within the shaft. The instrument with such a cross-sectional design are claimed to cut dentin more effectively and are composed of conventional NiTi alloy [7].

Whereas The PTN is a novel rotary file system and very rare reports on apical extrusion of debris resulting from its instrumentation. Capar *et al*., reported less debris extrusion associated with PTN files when it was compared to the universal Protaper file systems. The Protaper next possess a unique design, an offset center of mass and rotation. This design provides non uniform and reduced contact between the instrument and the root canal wall and also provides more cross-sectional space for enhanced cutting, loading and successfully allowing the debris to travel out of a canal (coronally), compared to a file with a centered mass and axis of rotation. It may also decrease the chances for the file packing the debris laterally, aiding in reducing the chances of blockage of the root canal system. This can be the main advantage of the protaper next file and may lead to least debris extrusion; the lower taper of the file may also lead to less debris extrusion. Hence, it was

used as one of the instrumentation techniques for the present study [14].

This methodology is generally accepted and has been used previously although the technique allows a comparison of the file systems under identical conditions, it does have limitations. The main disadvantage of the method is that vital periapical tissues cannot be mimicked. Apical extrusion was not limited, because of the absence of a physical backpressure provided by periapical tissues *in vivo*. This is an imminent shortcoming of *in vitro* designs with no periapical resistance; as a result certain degree of caution should be taken when transferring the present results to the clinical situation [15].

Results of this study indicate that practitioners should be aware about the extent of debris extrusion with each specific instrument system, which can probably be made the basis for selection of a particular instrument system.

CONCLUSION

Under the limitation of the study all hand and rotary instrumentation technique extruded bacteria.Protaper next file were was associated with less debris extrusion when compared to protaper universal and k files. Further invivo research in this direction could provide more insight in to the biological factors associated and focus on bacterial species that essentially play a major role in post instrumentation flare up.

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