Comparative study of apical extrusion of intracanal bacteria using different instruments and techniques (In vitro study)

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ABSTRACT

Background: To compare the number of bacteria extruded apically from extracted teeth after canal instrumentation using the nickel-titanium instruments (both Hand & Rotary ProTaper) and hand Stainless Steel instruments.
Methodology: sixty extracted single-rooted human teeth were used. Access opening was prepared and root canals were then contaminated with a suspension of Enterococcus faecalis. The contaminated roots were divided into three experimental groups of 20 teeth. Group 1, Rotary ProTaper group: the root canals were instrumented using Rotary ProTaper instruments. Group 2, Hand ProTaper group: the root canals were instrumented using Hand ProTaper instruments. Group 3, Hand Stainless Steel instruments group: the root canals were instrumented using Hand Stainless Steel instruments. Bacteria extruded from the apical foramen during instrumentation were collected into vials. The microbiological samples from the vials were incubated in culture media for 24 h. Colonies of bacteria were counted and the results were given as number of colony-forming units. The data obtained were analysed using the one-way ANOVA analysis of variance and t-tests p value 0.05 as the level for statistical significance.
Results: There was no significant difference to the number of extruded bacteria between the Rotary & Hand (P>0.05 Non significant) but there was significant difference between Rotary & Hand ProTaper and Hand Stainless Steel instruments (P < 0.05).
Conclusions: Hand Stainless Steel instruments extruded more bacteria apically than Rotary & Hand ProTaper.
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INTRODUCTION

A major objective in root canal treatment is to clean the root canal system. During the process dentine chips, pulp tissue fragments, necrotic tissue, microorganisms and intracanal irrigants may be extruded through the apical foramen. This is of concern as material extruded from the apical foramen may be related to post-instrumentation pain or to a 'flare-up' **(1)**.

Microbial irritants of pulp and periradicular tissues include bacteria, bacterial toxins, bacterial fragments, and viruses. These irritants egress apically from the root canal system into the periradicular tissues and initiate inflammation and tissue alterations. Oral bacteria involved in dental caries and endodontic disease are able to gain nourishment from tissue fluids. This may account for the presence of streptococci and enterococcci cases with post- treatment disease **(2)**.

Enteroccocus faecalis is the most commonly isolated species from the canals of teeth presenting post treatment disease [(2,3)] found E. faecalis in 52.94% of canals with bacterial growth. This microorganism has demonstrated the capacity to survive in an environment in which there are scant available nutrients and in which commensality with other bacteria is minimal.

During the last decade, root canal preparation with engine-driven nickel-titanium instruments has become popular. More recently advanced instrument designs including non-cutting tips, radial lands, different cross sections and varying tapers have been developed to improve working safety, to shorten working time and to create a greater flare within preparations **(4)**. This study was used to compare the amount of apically extruded intracanal bacteria by the use of Rotary Protaper, Hand Protaper and Hand SS files.

MATERIALS AND METHODS

Selection and preparation of teeth

All the teeth sterilized in an autoclave **(5)** and forced through a precut hole in a rubber stopper and placed into a larger 50-ml flask in order to avoid direct handling of the vial during instrumentation. A 27-gauge needle was placed through the stopper into the flask to equalize the

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pressure. A sheet of rubber dam was placed over the tooth to obscure the operator's view of the root and to prevent contamination of the vial during irrigation and instrumentation procedures 7.5ml from normal saline was added to the collection vial and 0.01ml of broth contains about $7.1x\ 10^5$ CFU was added to the canal of each sample by the use of the insulin syringe.

Sixty permanent human single rooted teeth were selected according to the following criteria: single canal with mature apices of the roots, no root caries or resorption, if there is any caries in the crown they were removed by bur in a high speed handpiece and restored by acid-etched composite resin to create a reservoir for loading a suspension of *Enterococcus faecalis.* Patent apical foramen in which size 10 file should pass through the apex without any resistance and size 15 file can not pass easily. Then, access opining was made for all the teeth and was sterilized in autoclave later. According to manufacturer's recommendations all instruments were used to enlarge five canals only. The teeth were divided into three experimental groups of 20 samples; each group containing similar numbers of the same tooth types with similar canal length, this ensured that the number of apically extruded bacteria was exactly to be due to the instrumentation technique and not to tooth morphology. Access opining was made for all the teeth by carbide fissure bur in high speed hand-piece, any pulp remnants were removed with a fine barbed broach and they were sterilized in autoclave later.

The working length was determined by placing size 15 file with a rubber stop carefully into each canal until it was just visible in the apical foramen. This length was noted and 1mm was subtracted to give the working length of the canal, all the selected teeth have a working length range between (19-23) mm.

According to manufacturer's recommendations Rotary Protaper and Hand Protaper instruments were used to enlarge five canals only. All files were visually inspected at 10X magnification prior to use to be sure that none of the files were distorted. If any distortions were found, that file was eliminated and copious irrigation with normal saline was performed repeatedly after the use of each instrument using disposable syringes and 27 gauge tips. Regardless of the technique used, all canals were irrigated with 1ml of normal saline after the use of each instrument by inserting the needle passively and never allowed to bind as the irrigant was being deposited into the canal[6]. A randomly laid down sequence was used to avoid bias towards one of the instruments. Only six samples were instrumented at a time to minimize operator fatigue and familiarity.

According to Schilder [7] who believed in limiting the apical enlargement to size 25 or 30 to minimize the undesirable effect like; ledging or zipping, due to decrease of the instruments flexibility with increase in its size, all canals were enlarged to an apical size 30. The sequences used in the present study were following the manufacturers' instructions for each system.

Group A: **Rotary ProTaper Ni-Ti files**:

ProTaper rotary endodontic files were set into permanent rotation (300 rpm) with a 16:1 reduction hand piece powered by a torque-limited electric motor using torque setting 1.2 Ncm. which is as stated by the manufacturers' instructions. Instrumentation was completed in a crown-down manner using a gentle in-and-out motion. Instruments were withdrawn when resistance was felt and changed for the next instrument.

- First step (S1) shaping file was used first and moved apically to 2mm short of the working length.
- Second step (SX) files were then used (4-5 mm from the working length).
- Third step (S1) to full working length for shaping the coronal two- third of the canal
- Fourth step (S2) to full working length for shaping the coronal two – third of the canal
- Fifth step finishing files (F1, F2, F3) sequentially to the full working length with only one pecking motion for each instrument.

Once, the instrument had negotiated to the end of the canal and had rotated freely, it was removed.

Group B: **Hand ProTaper Ni-Ti files**

These are a new non ISO instrument which has the same morphology as rotary ProTaper file. According to the main principle of the crown-down technique, it cut the canal walls by rotating clockwise with sufficient apical pressure until it engages the dentine, then rotate counter-clockwise to disengage and remove the file from the canal, repeat rotation motion until desired length is achieved. The instrumentation sequence was the same as group A.

Group C: Stainless steel files (control group)

In this group, canals were instrumented with hand stainless steel files and the step back technique described by Weine et al [8] was used, without Gates-Glidden drills to ensure that only the effect of the stainless steel files would be

evaluated after instrumentation. All instruments were used without pre-bending.

The step- back technique that was used in the present study involves the following.

- Determination of working length: by insertion of file #15 to 1mm shorter than the apical foramen.
- The sequence of the files as the following
  - # 10 to the working length.
  - # 15 to the working length.
  - # 20 to the working length.
  - # 25 to the working length.
  - # 30 to the working length.
  - # 35 to 1mm shorter than the working length.
  - # 30 to the working length.
  - # 40 to 2mm shorter than the working length.
  - # 30 to the working length.
  - # 45 to 3mm shorter than the working length.
  - # 30 to the working length.
  - # 50 to 4mm shorter than the working length.
  - # 30 to the working length.
  - # 55 to 5mm shorter than the working length.

  Recapitulation was made by size 10 to make sure that the apical foramen was not closed by dentine chips during instrumentation.

Counting of bacteria:

Input bacteria:

The bacterial count put inside the canal is 7.1 X 10^7 CFU/ ml (7.1 X 10^5 CFU/ 0.01) (original number O.N) of *Enterococcus faecalis*, according to pilot study the samples were left for about four hours in 37 ◦C to give time to bacteria to enter inside dentinal tubules. After that by insulin syringe we pushed air inside canal to remove the remnant of the broth from the canal.

Count of apically extruded bacteria:

After complete instrumentation the tooth stopper part were be removed and 1ml will be taken from collecting vial and we were use serial dilution from 10^{-1} up to 10^{-4} then culture was made and incubated in 37 ◦C aerobically for about 24 hours, then bacteria was counted in the form of colony forming unit (CFU).

RESULTS

The mean of V.C of apically extruded intracanal bacteria for the groups are presented in Table 1. Comparison of the mean number of extruded bacteria between R. ProTaper and S.S Hand files showed statistically significant differences ($P < 0.05$). There was a significant difference ($P < 0.05$) between H.Protaper and SS Hand files. However, the difference between R. ProTaper and H. Protaper groups was not statistically significant ($P > 0.05$).

Table 1: Comparison of V.C of apically extruded intracanal bacteria between the three instruments.

	Mean	SD	SE	Min	Max
H.P. V.C. X 10^4	20,642	5,751	1,286	9	30
R.P. V.C. X 10^4	18,566	10,828	2,422	3	36,75
Hand SS V.C. X 10^4	25,685	7,771	1,738	12,8	38,6

V.C= viable count

DISCUSSION

The main objective of root canal therapy is to prevent and treat periradicular inflammation by eliminating microorganisms from the root canal system and preventing subsequent reinfection [9] Many factors effect the amount of extruded intracanal materials such as; instrumentation technique, instrument type, instrument size and preparation end- point and irrigation solution [10,11]

When intracanal contents are pushed through, it will result in an antigen - antibody complex. This reaction will cause damage to the cell membrane resulting in prostoglandins release, bone resorption, amplification of the kinin system and ultimately pain for patient[12].

This study was conducted to determine apical extrusion of intracanal bacteria produced as a result of canal shaping; pulp tissue remnants were removed before preparation and had no influence on the results.

Enterococcus faecalis was chosen as the bacteriological marker in this study. It is a nonfastidious, easy to grow facultative aerobic bacterium of significant clinical importance that could be used in a study applying a bacteriological assessment method. Other bacteria commonly associated with endodontic infections may require symbiotic support from other bacteria, but E. faecalis has been reported to survive and successfully thrive alone[13].

E. faecalis has been implicated in persistent root canal infections and has been identified as the species most commonly recovered from root canals of teeth post-treatment disease[14].

All techniques are likely to extrude material through the foramen[10], but some will extrude a greater mass than others. The greater the amount of

debris and bacteria apically extruded the more sever the reaction. In all probability the situation in vivo is far more complex and involves not only the mass of debris but the type and virulence of bacteria bounded up in the debris and the resistance of host tissues[14].

In order to minimize the variables all the canals were instrumented by one operator (researcher), who was trained to all three techniques of instrumentations before starting the actual experimental work.

In this study the working length of the canal was 1mm short of the apical foramen as [15] demonstrated greater debris extrusion when canals were instrumented at a length where the file was observed to just protrude through the apical foramen versus 1mm short of the apical foramen also [11] reported that, when the instrumentation was performed to the apical foramen, significantly more debris was forced apically than when instrumentation was 1mm short.

As the debris can also be introduced into the periapical tissue during irrigating process, the passive injection of irrigants was used in order to reduce the debris forcing out of apical foramen with anything other than the preparation system being tested. Normal saline was used for irrigation because it has no antibacterial effect; in this way elimination and extrusion of bacteria depended on the mechanical action of the instruments[16]

The 27-gauge needle was used in this study because it was highly effective in reaching adequate depth during irrigation to remove debris that was left after instrumentation[16]. During the experimental work, the operator used rubber gloves during handling of the collection vials in order to avoid contamination of the vials also the outer flask and rubber dam sheet was used to prevent the contamination.

Rotary Protaper was used according to manufacture's instruction in a crown-down manner, According to the result of this study the engine-driven techniques extruded smaller amount of microorganisms. This result may be attributed to first; due to rotary motion, which tends to direct debris toward the orifice, avoiding it is compaction in the root canal[11]. As the greatest number of microorganisms in the root canal lie in the coronal third[17], initial preparation of this section of the root canal system helps to reduce the number of microorganisms that may be pushed apically. Early flaring of the coronal part of the preparation may improve instrument control during preparation of the apical third of the canal, this result in agreement with [18]. Second, NiTi files showed continuous taper with the largest canal diameter because increase taper of the NiTi files up to 19% where as SS instrument where only 2% tapers in addition the brushing action that is recommended with ProTaper systems before further advancing the instruments which may caused to remove more debris coronally. Similar have been also established by several studies [19].

Third, narrower diameter at the apical foramen with SS files when compared with Protaper due to high rigidity of stainless steel file if compared with flexible NiTi instrument.

Hand Protaper instruments was used according to manufacture's instruction in a crown down technique, there was no statistical difference between the hand and engine-driven NiTi protaper instruments in terms of extrusion of microorganisms. This result because protaper for hand use have the same design, philosophical fundamentals of preparation, indications and sequence of rotary protaper. There was Significant difference between hand protaper and hand SS.

Hand Stainless Steel files was used in step-back technique and according to the result of the study the large amount of material extruded by step back occurred probably due to filling motion used during instrumentation of the apical third. The filling action of the instrument may act as a piston, pumping the irrigation solution and debris through the apex. It can also explain the limited extrusion produced by the engine-driven techniques which use alternate rotary motions. The results of this study showed that all endodontic instruments and canal instrumentation technique used was extruded intracanal bacteria apically.

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