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To Study the Efficacy of Reciprocating Single File System and Multifile Rotary Systems in Elimination of Root Canal Bacteria and Endotoxins

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AIM: To study the efficacy of reciprocating single file system and multifile rotary systems in elimination of root canal bacteria and endotoxins.

MATERIALS AND METHOD: Forty permanent single rooted mandibular premolars with straight canals inoculated with Escherichia Coli suspension for 21 days were selected for the study. Teeth were randomly divided into four groups (n=10) according to instrumentation system: Group A–Reciproc (VDW); Group B–WaveOne (Dentsply Maillefer); Group C–MTwo (VDW); and Group D–K₃ (Sybron Endo); Bacterial and endotoxin samples were collected with a sterile paper point before instrumentation and after instrumentation. Culture methods estimated the colony-forming units (CFU) and the Limulus Amebocyte Lysate test was used for quantification of endotoxins. Results so obtained were calculated and statistically analysed.

RESULT: Results before instrumentation concluded that bacteria and endotoxins were found in all of the investigated root canals. After instrumentation all systems were associated with the significant reduction of the bacterial load and endotoxins respectively: Group A–Reciproc (88.25% and 89.10%); Group B– WaveOne (83.53% and 80.59%); Group C– MTwo (79.41% and 75.84%) and Group D– K₃ (67% and 74.4%). Statistically no difference was found amongst the instrumentation systems regarding bacteria and endotoxin elimination (P <0.05).

CONCLUSION: The reciprocating single file, Reciproc and WaveOne were as effective as the multifile rotary systems for the eradication of bacteria and endotoxins from root canals.

KEY WORDS: Endotoxin, Waveone, Reciproc, Colony Forming Unit, Limulus Amebocyte Lysate test.

INTRODUCTION

Bacteria and their end products mainly endotoxins are responsible factors for causing pulpal and periradicular pathologies. Root canal system is inhabited by different types of microflora but gram-negative bacteria are predominantly responsible for endodontic infections.¹ Endotoxins are released from outer cell membrane of gram- negative bacteria after multiplication of non-viable bacteria.

Clinical studies conducted at the molecular levels have revealed that although cleaning and shaping significantly decreased the microbial load in canals, various cases still indicated the presence of infection after root canal preparation.²

In the past few years, endodontics has flooded with innumerable advancements and technological implementations and applications with the focus of enhancing the prognosis of therapy, increasing the contribution to the preservation as well as increasing the longevity of teeth.³ One of the new advancement that stand out is rotary instrumentation made of nickel-titanium components, featuring the ability to afford a uniform preparation in less time, thus

reducing work fatigue and stress imposition on the patient.⁴

Automated rotary systems are advantageous in comparison to the manual techniques, in terms of rapid and more centred preparations.⁴ These advantages and superiority of rotary systems were still not enough to avoid instrument fatigue and separation rates, a new concept of instrumentation was proposed known as reciprocation⁵, that was based on alternating movement.

LAL test is highly specific and sensitive key available for the detection and measurement of bacterial endotoxins, commonly called as Pyrogen. An enzyme-mediated response to endotoxin causes blood coagulation in Limulus Polyphemus. The rate of reaction depends on amount of endotoxin, the pH and the temperature. The lysate provides bivalent cations, a proclotting enzyme system and clottable protein required in the reaction.⁶

Therefore, the study aims to evaluate the effectiveness of single file reciprocating systems for



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the elimination of bacteria and endotoxins from root canals in comparison with the multifile rotary systems.

MATERIALS AND METHOD

Study was conducted in the department of Conservative Dentistry and Endodontics, Bhojia Dental College and Hospital, Baddi (H.P). Approval was granted by Ethical Committee. Forty permanent single rooted mandibular premolars with straight canal were selected for the study.

All teeth were decoronated with sterile diamond discs to maintain standardization (15 mm). Canal patency was checked by placing #15 K-file 1mm beyond the apical foramen. The teeth were placed in 17% EDTA followed by 5.25% Sodium Hypochlorite for 10 minutes each for opening the dentinal tubules for easy penetration of Escherichia Coli. After that, root canals were washed with phosphate buffered saline followed by distilled water to remove residues of the EDTA and Sodium Hypochlorite. All the teeth were autoclaved at 121°C and 15psi for 20 minutes. Culture broth was prepared by inoculating pure culture of Escherichia Coli to freshly prepared sterile BHI broth and followed by incubation at 37°C for 24 hours. Before inoculation, canal patency was re-checked with a sterilized #15 K-file. The samples were inoculated with 10µl of the prepared Escherichia Coli suspension BHI broth. The inoculated samples were incubated at 37°C for 24-48 hours.

The infected samples were equally divided into four experimental groups as per the instrumentation technique:-

1. **Group A (n=10):** Teeth were prepared with Reciproc file
2. **Group B (n=10):** Teeth were prepared with WaveOne file
3. **Group C (n=10):** Teeth were prepared with MTwo file
4. **Group D (n=10):** Teeth were prepared with K3 file.

The first endotoxin sampling was done by placing sterile paper points (size #15, Dentsply Maillefer) into the full length of the canal and retained in position for 1 minute. Immediately thereafter, the sample was put in pyrogen free vial containing 1ml of BHI broth. After the first sampling, the root canals were instrumented according to the group. All instruments

were set into permanent rotation with a 6:1 contra-angle handpiece (X Smart plus, Dentsply Maillefer) powered by a torque-limited electric circuit. For each MTwo and K3 file systems, individual torque limit and rotational speed programmed in the file library of the motor were used, whereas Reciproc and WaveOne were used in a reciprocating working motion generated by the motor.

Regardless of the technique used, canals were flushed with 0.9% normal saline solution in between instrumentation and care was taken that a standardized amount of irrigant (30 ml) was used during each canal preparation.

Serial dilutions were made with the sample containing BHI broth from 10³-10⁶ and triplicate plating of sample was done to look for CFU/ml for bacterial colonies and the 1ml of sample from each dilution from 10³-10⁶ was put in LAL reagent and looked for color change which was assessed by Elisa plate reader at 405 nm. Quantitative estimation of endotoxin was then done using standard graph provided in the LAL kit (Thermofisher Co.).

RESULTS

The percentage values of bacterial and endotoxin reductions found in all groups tested after instrumentation are shown in Figure 1. Endotoxins were detected in all the samples collected from the root canals. No statistical differences were found in the percentage values of endotoxin reduction obtained between reciprocating systems (Reciproc [89.10%], WaveOne[80.59%]) and multifile rotary systems (MTwo [75.84%] and K3 [74.4%]) (P < 0.05). In the initial samples, bacteria were recovered from all of the tested root canals. Both reciprocating systems: Reciproc [88.75%]) and WaveOne [83.53%] and multifile rotary systems: (MTwo [79.50%] and K3 [67%]) showed similar efficacy for eliminating bacterial load (P < 0.05).

DISCUSSION

The success of endodontic treatment is solely dependent upon complete elimination of microorganisms and their endotoxins from root canal. Induction of inflammation and bone resorption caused by bacteria and endotoxins lead to initiation, propagation and persistence of periradicular lesions.^{1,7} The microbial load within an infected root canal varies from 10²-10⁸ according to Sjogren et al., 1991.⁸ Microbes invade all parts of root



Figure 1. Comparative counts of Colonies of E.coli (CFU/ml.) before and after treatment

Instrumentation Group	CFU count after treatment	Percentage reduction in CFU count	Endotoxin level after treatment	Percentage reduction in Endotoxin level
Reciproc	8.50-16	72.93%-88.75%	13-24.68	78.51%-89.10%
WaveOne	11.3-16	73.56%-83.53%	24-35	72.93%-80.59%
MTwo	7-22	65.50%-79.41%	31-39	68.88%-75.84%
K3	7.5-25	44.79%-67%	37-54	58.94%-74.4%

Table 1. Mean percentage reduction in CFU count and endotoxin level using different instrumentation techniques

S.no	Title	Chi square value	p value
1.	Percentage bacterial reduction	116.000	.430*
2.	Percentage endotoxin reduction	85.333	.530*

Table 2. Statistical Analysis (*p <0.05 significant)

canal system and they are found at depths upto 300µm in dentin which makes their elimination very difficult.⁹ Since last decades endodontics has seen various advancements in terms of technological means focusing on the preservation of natural dentition.¹⁰

Introduction of NiTi alloy in endodontics in the year 1988 lead to development of rotary systems with the objective of simplicity in addition to reduction in stress levels.¹¹ NiTi instruments are available in different tapers and designs resulting in faster, safer and better quality preparations. Use of multifile system having different tapers results in significant

reduction in microbial number but is time consuming process. Recently, single file reciprocating systems have been introduced in dentistry which focuses on root canal preparation with single file only that claims better resistance to instrument separation permitting easier treatment.

The samples were decoronated at the length of 15 mm from cementoenamel junction to maintain standardization of specimens. The root canals were prepared with # 25 WaveOne (8% taper), # 25 Reciproc (6% taper), # 30 K3 (6% taper) and #30 MTwo (6% taper) respectively in crown down technique. Crown down technique was followed because it permits straight line access to apical region, removes coronal interferences and also provides the advantage of deeper penetration of irrigants.¹²

In the present study, the Reciproc and WaveOne files (#25) were used composed of NiTi alloy known as M-wire made by innovating thermal treatment.¹³ These two files were compared with K3 NiTi file system which is third generation rotary system (#25, taper 0.06) and MTwo NiTi rotary system (#25, taper

0.06) having taper ranging from 0.06-0.07 which is similar to Reciproc and WaveOne. The chromogenic LAL assay used in the study is preferred for the analysis of endotoxins in root canal infection because of its precision.

Bacterial culture analysis depicted the presence of bacteria in all initial canal samples, with the CFU values per canal ranged from 9×10^5 - 70.8×10^5 which were comparable to the results reported by Xavier et al., 2013¹⁴, Martinho et al., 2014¹⁵ and Martinho et al., 2008.¹⁶ Pre-instrumented samples from the root canal revealed the level of endotoxin ranged from 112.3 -173 EUs/ml for both in vivo and in vitro studies which is comparable to the studies conducted by Martinho et al., 2014¹⁵, Martinho et al. 2010¹⁷ and Xavier et al., 2013.¹⁴ The culture analysis revealed significant bacterial reduction in both single file reciprocating instruments i.e Reciproc (88.94%), WaveOne (83.09%) and in multifile system i.e MTwo (75.57%) and K3 (76%). Present study showed better bacterial reduction with reciprocating file systems as revealed by Alves et al.¹⁹ However, no statistical difference was found amongst different files which are comparable to the study conducted by Burklein et al., 2012.¹⁸ The percentage value of endotoxin reduction achieved between single file Reciproc (88.75%) and WaveOne (83.57%) and multifile rotary system MTwo (79.41%) and K3 (67%) showing better elimination with reciprocating systems which were in accordance with the study conducted by Alves et al.,2012¹⁹ however no statistical difference was found amongst different files. These findings were similar to the results reported in vitro study conducted by Machado et al., 2010¹⁰. Alves et al.,2012¹⁹ revealed significant reduction in microbial load by using single file systems.

With regards to the percentage reduction level of endotoxin after instrumentation both single file reciprocating instruments : Reciproc (91.01%) and WaveOne (87.75%), and multifile file systems MTwo (77.44%) and K3 (72.05%) better reduction in endotoxin levels was shown by reciprocating systems but no statistical difference was found amongst the different files systems used which are in accordance with the study conducted by Martinho et al., 2014.¹⁵

It is highly important to highlight that the reciprocating instruments considered (WaveOne

and Reciproc R25 files) had a tip diameter equivalent to a #25 K file. Conversely, in the Mtwo and K3 systems, the final instruments used for canal preparation had a tip diameter of #30 K file. In addition, the WaveOne file 25 has a continuously decreasing taper from the tip to the shaft (o.o. 0.65, 0.60, 0.55) having different cross-sectional designs over the full length of file, but in the middle part modified triangular convex cross-section with radial lands changes to a neutral rake angle with a triangular convex cross-section near the shaft according to Burklein et al., 2012.¹⁸

Despite of variations in apical preparation diameter and differences in the design characteristics of the file systems tested in the study, results showed no differences in the median percentage values of bacterial and endotoxin reduction after culture analysis and endotoxin estimation. Moreover, none of the file system completely eliminated bacteria and endotoxin from the root canal.

CONCLUSION

No file system established root canals free from bacteria and endotoxins. Thus, root canal disinfection should not be concentrated only on a mechanical preparation or on the selection of the instrument sequence, but mechanical preparation of root canal must be assisted by appropriate irrigation protocol leading to successful endodontic treatment.

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