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EVALUATION OF APICAL EXTRUSION OF INTRACANAL BIOFILM AFTER ROOT CANAL PREPARATION USING THREE DIFFERENT ROTARY INSTRUMENTATION SYSTEMS: AN IN VITRO STUDY

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ABSTRACT

Background- Endodontic instrumentation carries the risk of extrusion of debris and bacteria. The instrumentation technique used and the type of instrumentation influences this risk of extrusion.

Aim- The purpose of this study was to evaluate and compare the apical extrusion of intracanal biofilm after root canal preparation using Hyflex CM, K3XF and Heroshaper.

Objective- 1)To evaluate the amount of bacteria extruded apically after root canal preparation using Hyflex CM.
2)To evaluate the amount of bacteria extruded apically after root canal preparation using K3XF.
3)To evaluate the amount of bacteria extruded apically after root canal preparation using Heroshaper.
4)To compare the amount of bacteria extruded apically after root canal preparation using Hyflex CM, K3XF, Heroshaper.

Methodology- Sixty five extracted single-rooted human mandibular premolar teeth were used. Endodontic access cavities were prepared and root canals were then contaminated with a suspension of Enterococcus faecalis and dried. Re-inoculation process was done upto 30 days.The contaminated roots were divided into five groups. Group 1=Hyflex CM,Group 2=K3xf,Group 3=Heroshaper,Group 4= Negative Control Group,Group 5= Positive Control Group.Before instrumentation positive control group was checked by SEM.Group 1,2 and 3 were instrumented upto #25,4%.Bacteria extruded from the apical foramen during instrumentation were collected into vials.Samples were incubated in brain heart incubation agar at 37^0 C for 24 hr.The resulting bacterial titre ,in colony-forming units(CFU) per ml was determined. The data obtained were analysed using the Analysis of variance (ANOVA) Test and post hoc analysis.

Results- Heroshaper extruded least amount of bacteria followed by K3XF files. Maximum extrusion of E. faecalis was seen in hyflex CM group.

Conclusion- All the rotary instruments extruded bacteria apically after root canal preparation.

INTRODUCTION

Successful endodontic treatment depends on various factors such as diagnosis, access preparation, chemomechanical preparation, disinfection and sealing of the root canal walls, etc. Biomechanical preparation is an important stage in endodontic therapy. During biomechanical preparation dentin chips, pulp tissue fragments, necrotic tissue, microorganisms, and irrigants may be extruded apically. Extrusion of microorganisms and their byproducts apically during root canal instrumentation may cause a delayed healing process and endodontic flare-up (Seltzer & Naidorf 1985).[1] It may generate an acute inflammatory response the intensity of which depends on quantitative factor and qualitative factors. Dummer(1995) results that the quantitative factor(the number of bacteria) is to be under the control of the clinician and the qualitative factor(virulence factor) is difficult to control.[2]

Endodontic infections are caused by several types of microorganisms. It may be a primary or secondary endodontic infection. The microorganisms seen in primary endodontic infection are Bacteroides, Prophyromonas, Prevotella, Fusobacterium, Treponema, Peptostreptococcus, Eubacterium And Campylobacter species and the microorganisms seen in secondary endodontic infection are Enterococci, Streptococci, Lactobacilli, Actinomycetes and Fungi. Some species are resistant to antimicrobial treatment and can survive in the root canal after root canal preparation. The most common species are Fusobacterium nucleatum, Prevotella, Campylobacter, E. faecalis, Streptococci, and Lactobacilli. E. faecalis species are commonly found in post-endodontic infections. Biofilm is defined as a sessile multicellular microbial community characterized by cells that are firmly attached to the surface of the root canal wall and embedded in a self-produced extracellular matrix.[3] The matrix takes 85% of the volume of a biofilm.[6] E. faecalis species can survive in the presence of medicated canal(e.g-calcium hydroxide) and irrigant(e.g-sodium hypochlorite).[4] E.faecalis species have the ability to form a biofilm in the medicated root canal. Some studies have shown that E.faecalis has the ability to associate with F.nucleatum and promote endodontic infection.[5] Extrusion of intracanal microorganisms and infected debris has been reported with all instrumentation techniques, although working length is maintained.Recent improved instrument designs have been introduced to improve working length safety and to create a greater flare within preparations.[1]The purpose of this study was to evaluate and compare the number of bacteria extruded apically after root canal instrumentation by using Hyflex CM, K3XF, and Heroshaper.

Materials and method

Sixty-five (65) extracted human single-rooted permanent mandibular premolars were collected.The inclusion criteria of this study is single rooted human mandibular permanent premolars in which the root has been formed completely,teeth with no cracks or fracture, no root caries, no resorption, no calcification.The exclusion criteria of this study is teeth with root caries, immature apex, and endodontically treated teeth.All teeth were stored in a normal saline solution. The teeth were thoroughly cleaned with an ultrasonic scaler to remove calculus, stains, or any tissue remnants on the roots. Pre-operative radiographs were taken in buccolingual and mesiodistal directions. Endodontic access cavities were prepared using Endo Access Bur then a size #10K file was used to establish the canal patency.

TEST APPARATUS

Holes were created in the center of the rubber stopper of a glass vial with the hot instrument. Then each tooth was inserted into the center of each rubber stopper and fixed at the level of CEJ. Before insertion, the teeth were applied with two coats of nail varnish on the external surface of the root to prevent bacterial microleakage through lateral canals. Then a 24 gauge needle was placed alongside the rubber stopper to equalize the air pressure inside and outside the glass vial. Then the glass vial was filled with 10 ml NaCl solution.

CONTAMINATION WITH E.faecalis BIOFILM

Cultured E. faecalis strains were incubated aerobically. Using a sterile micropipette, bacterial suspension was transferred to the root canal and then stored at 37 ^{0}C for 24 hr. Re-inoculation process was done for upto 30 days under the biosafety cabinet. All the samples were divided into 5 groups. Before instrumentation positive control group(n=5) was checked by SEM whether the biofilm was still viable after 30 days. After confirmation #10 K file was placed in the canal upto the apical foramen. Then the root canal instrumentation was done 1mm short of the working length and all root canals were irrigated with 3ml of 3% NaOCL

GROUP 1 Hyflex CM file- According to manufacturer instructions, instruments were used at a speed of 500 rpm and 2.5 Ncm torque at the WL. Files were used sequentially size 25,0.08 for coronal flaring then sequentially used of size 20,0.06 to the WL for shaping the coronal two-thirds of the canal. The apical one-third was finished by using 20,0.04 followed by size 25,0.04 to the WL.

GROUP 2 K3XF file- According to manufacturer instructions, instruments were used at a speed of 350 rpm and 2.9 Ncm torque at the WL. Files were used sequentially sizes 25,0.10 and 25,0.08 for coronal flaring. The 25,0.06 was used for shaping the coronal two-thirds of the canal and the apical one-third was finished by using 25.0.04 up to the WL.

GROUP 3 Heroshaper- According to manufacturer instructions, instruments were used at a speed of 450 rpm and 1.2Ncm at the WL. Size 25,0.06 were used for shaping two-thirds of the root canal then sequentially used size 25,0.04 up to the WL.

GROUP 4 Negative control group- uninstrumented group.

GROUP 5 The Positive control group was checked by SEM whether the biofilm was viable after 30 days.

At the end of canal preparation, 1ml NaCl solution was taken from each experimental vial, which was then plated on brain heart infusion agar and incubated at 37 ^{0}C for 24 hr. The resulting bacterial titer in colony-forming units(CFU) per ml, then was determined.

Figure 1:Test Aparatus

STATISTICAL ANALYSIS

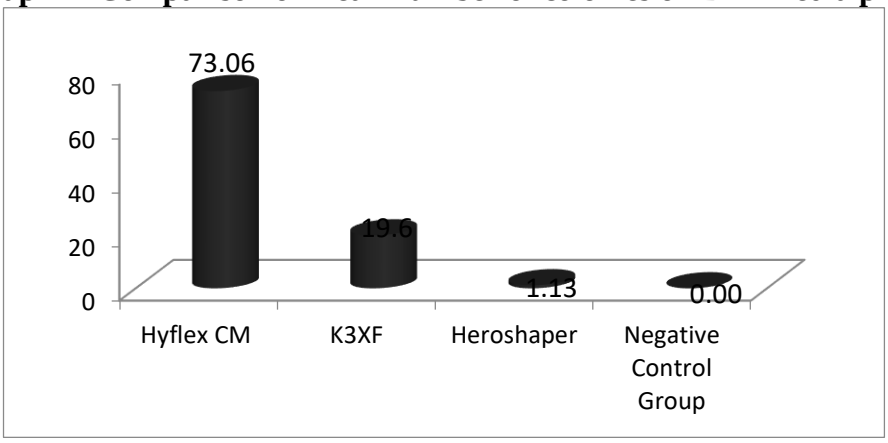
Collected data were entered into Microsoft Excel and subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS, IBM version 20.0). 5% was chosen as the significance level and statistical significance was defined as a value of $p \leq 0.05$.The Kolmogorov-Smirnov test and the Shapiro-Wilks test were used to measure data normality.ANOVA and post hoc analysis were performed for quantitative variables.

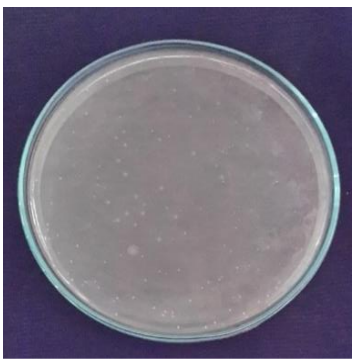
RESULTS

The results are based on in-vitro analysis of 65 samples comparing apical extrusion of intracanal biofilm after root canal preparation using Hyflex CM, K3XF, and Heroshaper, a positive and negative control group. The results of positive control were not taken in the analysis because this group was

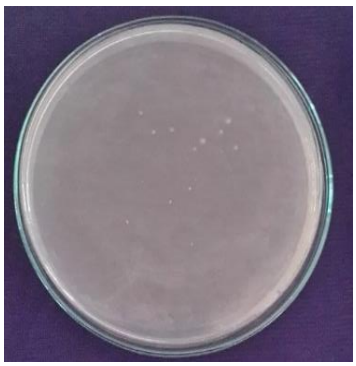
checked with SEM whether the biofilm was viable after 30 days.Heroshaper files showed the least apical extrusion of bacteria followed by K3XF and Hyflex CM files. Maximum bacterial extrusion was seen in the hyflex CM group and no extrusion was seen in the control group.

Graph 1- Comparison of mean number of colonies on BHI media plate





Group I



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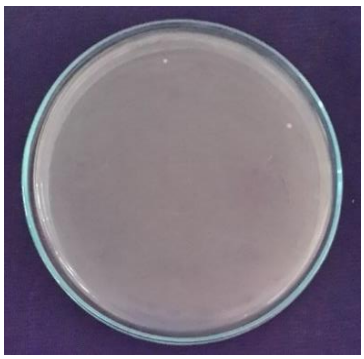
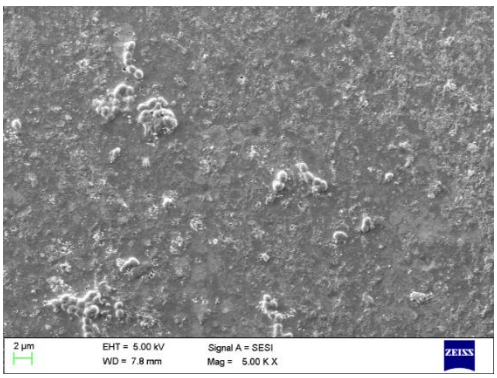


Group III

Group IV



Group V

DISCUSSION

Biofilm is a mode of microbial progression in which dynamic communities of interacting sessile cells are irreversibly connected to a solid substratum as well as to each other and are embedded in a self-made matrix of extracellular polymeric substances.[5]It is a thin layered condensation of microbes that can include bacteria, fungi, and protozoa. Dental caries, gingivitis, periodontitis, peri-implantitis, and apical periodontitis are caused due to the formation of microbial biofilm rather than single micro-organisms. Bacteria present in the oral cavity serve as a primary source of biofilm formation within the root canal system.[6]E.faecalis species is one of the most common bacteria seen in the case of failed root canal therapy and causes persistent infections.In our study, E. faecalis has been used as a bacteriological marker because it is the most frequently isolated microorganism and is usually seen in

root canal infections. Microbiological findings from endodontically treated teeth with the persistent periapical disease have shown a high ratio of enterococci species.[7]It is a non-fastidious bacteria and can form a biofilm. They can live and persist in poor nutrient environments. Enterococcus can suppress the action of lymphocytes and potentially contribute to endodontic failure.[5]Apical periodontitis is a group of inflammatory diseases caused by bacteria infecting the necrotic root canal system.[8]The primary objective of endodontic treatment is cleaning, shaping, and sealing of the root canal system. Biomechanical preparation of the root canal is an important aspect of endodontic treatment. Apical extrusion is one of the most common problems experienced during biomechanical preparation (Van der Sluis,2006; Gu,2009; Seltzer,1985). This may have adverse effects on the prognosis of the root canal treatment as it may lead to flare-ups.A flare-up can be characterized by the occurrence of pain or swelling on the facial soft tissues and the oral mucosa in the area of the endodontically treated tooth that occurs within a few hours or days during root canal therapy.[9]Some of the studies reported that the varying frequencies of flare vary from 1.4% to 16%(Morse et al .1986, Torabinejad et al,1988, Barnett &Tronstad 1989, Siqueira et al. 2002) and up to 50% (Oliveira Alves V. 2010).[10]

Various factors such as mechanical, chemical, or microbial injury to periarticular tissue during root canal instrumentation can cause degranulation of mast cells. The intensity of the tissue injury response depends on the type of injury and intensity of the inflammatory response. Walton and Fouad concluded that the frequency of flare-ups is significantly higher in necrotic pulp cases than in vital pulp cases. Apical extrusion of infected debris or bacteria to the periradicular tissue during biomechanical preparation is one of the major causes of post-operative pain.[10]

Postoperative flare-up and periapical healing depends on the amount and the type of debris or bacteria extruded apically after root canal instrumentation. Many of the authors measured the amount of intra-canal bacteria extruded apically after root canal preparation through microbiological culture.[11]

There are various methods to detect the intracanal biofilm in infected root canals such as scanning electron microscopic (SEM) image, confocal laser scanning microscopic(CLSM) image, atomic force microscopy(AFM), transmission electron microscopy(TEM) .[12]In the present study, SEM evaluation have been used in group 5 i.e positive control group.

In the present study, single-rooted permanent mandibular premolars were selected because the presence of more than one canal may affect the final amount of bacteria extruded apically during root canal instrumentation.[13]

Sodium hypochlorite (NaOCl) is one of the most common irrigating solution used in endodontics. Sodium hypochlorite is used as an endodontic irrigant because it has effective antimicrobial and tissue-dissolving capabilities.0.5% - 5 % conc. is commonly used. Some studies have shown that instrumentation and irrigation with NaOCl would eliminate bacteria in 50-75% of the infected root canal at the end of the first treatment session.[5]In our study,3% NaOCl was used as an irrigating solution during root canal preparation.

Various instrumentation techniques are efficient for cleaning and shaping like step back, crown down and hybrid technique. According to various research data, all the instrumentation techniques have shown some amount of intracanal debris or bacteria extruded into the periradicular area. Nowadays NITI instrument systems have been popular for root canal preparation.[14]Our study Hyflex CM, K3XF and heroshaper has been used.

Various factors may affect the amount of extruded intracanal materials such as instrumentation technique, irrigation solution, and instrument type and size.In the present study, the apical diameter of the master apical instrument in Group 1, Group, and Group 3 was standardized at size 25.4% to avoid any variations in the amount of extruded intracanal bacteria due to the size of apical enlargement.[2]

Hyflex CM(Coltene Whaledent) was introduced by Ricardo Caiecedo & Stephen Clark in 2011 it was machined from a wire termed CM-wire with double fluting, symmetrical cross-section, variable pitch, noncutting tip, absence of radial land, negative rake angle. It was manufactured in a special thermomechanical procedure which has to increase the flexibility of a file. The controlled memory

effect helps the file retain the shape of the canal even when it is out of the canal. This feature is responsible for reducing the risk of lodging, transportation, or perforation. Approximately 90% of Hyflex CM instruments undergo plastic deformation during manufacturing but return to their initial condition once autoclaved.[15,16]

The Heroshaper is a second-generation nickel-titanium rotary instrumentation system. The number of instruments is reduced to 2 from 3, and their tapers are 2%,4%, and 6%. Hero shaper instruments have triple helix cutting edge, adapted pitch, and positive rake angle. The purpose of this feature is to prevent the instrument from binding in the root canal. The helical angle of the cutting edges varies from the tip to the shank.[17]

In our study, the Hyflex CM file showed more extrusion of bacteria as compared to the hero shape. There is no data until now, on apical extrusion of intracanal bacteria after root canal instrumentation by heroshaper file but there is data available on debris extrusion. According to Reddy et al (2017), the hero-shaper rotary system showed the minimum amount of debris extrusion than the Mtwo file because glide preparation and crown down preparation could be the probable reason for less debris extrusion.[18]According to Walsch, the files with helical cutting edge, positive rake angle along with adapted pitch have better dentin cutting efficiency and debris removal from the root canal system.[19] This design feature is similar to the hero shape. Jale Tanalp et al in 2006, conducted a study on the amount of debris extruded apically after root canal instrumentation when using ProTaper, ProFile, and HERO Shaper. Based on their results, pro taper showed a higher amount of debris extrusion than profile and hero shape. In this study,3 instruments have been used for apical preparation in the protaper group and 2 instruments have been used in profile and hero shape. The use of less number of instruments during root canal preparation may affect the amount of debris extrusion. This could be a probable reason for less extrusion of bacteria in hero shapers.[17]

Sonali Taneja et al in 2015 compared the apical extrusion of e.faecalis after instrumentation with hyflex CM, GTX, and protaper. Based on her results, hyflex CM extruded a greater amount of e.faecalis after root canal instrumentation than the GTX file. This may occur due to the absence of radial land and because of the increase in the number of files used during root canal instrumentation.[20]The result of the above study was similar to our study which showed that the hyflex CM extruded a greater amount of e.faecalis after root canal instrumentation.

K3XF is the next generation of K3 files and is manufactured with R-phase technology. This file system has variable pitch and, positive rake angle with asymmetrical radial land. It has superior flexibility and resistance to cyclic fatigue.[21]

Recai Zan et al 2017 conducted a study on apical extrusion of intracanal biofilm using WaveOne Gold, ProTaper Gold, Twisted File Adaptive, OneShape new generation and K3XF.Based on the results wave one gold and proper gold extruded less amount of bacteria apically than one shape new generation, twisted file adaptive, and K3XF. This may occur due to metallurgy, design features, and kinematics of these systems. It was concluded that Gold systems may be preferred as safer to minimize the apically extruded bacteria during root canal treatments.[22]In the present study, the Hyflex CM file showed more extrusion of bacteria as compared to K3XF. The amount of bacteria extrusion observed for K3XF in this study i.e. 19.6 ± 3.9 is close to the value observed by Recai ZAN et al. (2017) i.e. 17.12 ± 2.67. This may occur due to the absence of radial land.

Another study was conducted by Recai et al in 2016 where they compared the amount of intracanal bacteria extruded apically during instrumentation using Revo-S, Twisted file adaptive, One shape new generation, Protaper next, and K3XF. Based on the results, the amount of bacteria extrusion apically is less in K3XF and OSNG as compared to revo-s, twisted file adaptive, and protaper. This may occur due to different designs of blades, flutes, helical angles, and pitch shapes. The amount of bacteria extrusion observed for K3XF in the present study i.e. 19.6 ± 3.9 is similar to this study i.e. 17.15 ± 3.41.[23]

According to Priyanka Ghogre et al 2015, they concluded K3XF showed less amount of bacterial extrusion amongst all groups. This may occur due to its unique design feature of variable pitch which helps to prevent the screwing effect of the instrument.[24]

Initial preparation of the coronal third of the root canal system helps to reduce the number of microorganisms that may pushed apically because more microorganisms are present in the coronal third of the root canal. Early cervical flaring of the root canal may also improve instrument control for the preparation of the apical third of the root canal. The advantages of early flaring are deeper penetration of irrigating solutions, easy removal and clearance of debris from apical third, and reduced possibility of lodging and debris packing.[25] In our study, the crown down technique was used with Hyflex CM, K3XF, and Heroshaper Ni-Ti rotary instruments.

There has been no study reported to date comparing the extrusion of intracanal bacteria by Hyflex CM, K3XF, and Heroshaper files. The results of this Invitro study cannot be compared with the Invivo study. It should be noted that, unlike the invitro environment, the presence of periapical tissues in clinical conditions creates back pressure, which prevents the entry of debris and intracanal bacteria. It is a fact that a practitioner should be extra careful in the instrumentation of severely infected root canals. Further investigations in this direction may give further insights into the biological factors associated with the correlation and consequences of apically extruded bacteria. Therefore furthermore studies are required in this field.

CONCLUSION

Within the limitations of this study, it was concluded the instrument systems, Hyflex CM, K3XF, and Heroshaper extruded intra-canal bacteria apically. Hyflex CM extruded more bacteria apically than K3XF and Heroshaper. Heroshaper extruded the least bacteria apically after root canal preparation.

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