Microbiological Assessment of Root Canals Following Use of Rotary and Manual Instruments in Primary Molars

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Aim: *To assess the microflora of root canals in primary molars following use of rotary NiTi files and conventional hand NiTi and stainless steel files.* ***Study design***: *This randomized clinical trial consisted of a total of 60 first and second primary molars requiring root canal treatment, who were selected from children aged 5-9 years. Based on type of root canal instrumentation, the teeth were randomly assigned to three groups of twenty teeth each; Group A: Rotary NiTi files, Group B: Hand NiTi files and Group C: Hand stainless steel files. Following administration of local anesthesia, isolation with rubber dam was carried out. For the purpose of instrumentation and sampling, the palatal canal of maxillary molars and the distal canal of mandibular molars were selected. Prior to sampling, the orifices of other canals in these teeth were sealed, so as to prevent any contamination. Instrumentation was carried out in each group using respective instruments along with intermittent saline irrigation. Root canal samples were obtained both before and after instrumentation, using sterile absorbent paper points and transferred to a sterile vial with transport fluid. Serial dilutions were prepared and cultured on suitable agar media. Both aerobic and anaerobic microbial counts were made. Data obtained was subjected to statistical analysis using Wilcoxon signed rank test and one-way Analysis of variance.* ***Results***: *In all three groups, there was a significant reduction in both aerobic and anaerobic mean microbial count following root canal instrumentation. ($p<0.001$).* ***Conclusion***: *Rotary NiTi files were as efficient as conventional hand instruments in significantly reducing the root canal microflora.*

Keywords: *instumention, microflora, Rotary, NiTi*

INTRODUCTION

Pediatric endodontic treatment has shown great development in recent years. There has been an improvement not only in the materials but also in instrumentation techniques. The primary objective of cleaning and shaping the root canal system is the removal of vital and/or necrotic pulp tissue, infected dentine, and dentine debris in order to eliminate the bulk of micro-organisms present in the root canal.[1]

Root canal preparation in primary teeth has been routinely carried out using hand instruments, which are time consuming and may lead to iatrogenic errors such as ledging and perforation.[2] The development of nickel titanium instruments and the possibility of changing the traditional design and taper led to the introduction of rotary endodontics. Studies have confirmed that NiTi rotary systems are faster than hand instruments, eliminate problems during preparation of curved root canals and result in better conservation of the tooth structure.[1,3,4-7] Rotary NiTi instruments have been shown to have better cleaning ability than hand files or at least yielded the same results.[2,6-8]

The introduction of the rotary system with Ni-Ti files for preparation of primary teeth is recent. Studies on the ability of rotary instruments to reduce intra-canal flora in primary teeth are lacking. Therefore, the present study was undertaken to evaluate efficacy of rotary nickel-titanium files in reducing microflora in root canals of primary molars, in comparison to manual instrumentation.

MATERIALS AND METHOD

Healthy and co-operative children, with no history of prolonged systemic illness were selected from patients attending the Department of Pedodontics and Preventive Dentistry. Ethical clearance to conduct this randomized clinical trial was obtained from the institutional review board. Following clinical and radiographic examination, a total of 60 first and second primary molars (maxillary and mandibular) requiring root canal treatment were selected from children aged 5-9 years.

Inclusion Criteria: Tooth with carious pulp exposure, diagnosed as having irreversible pulpitis, tooth showing radiographic signs of pulpal or interradicular involvement ranging from slight thinning of the trabeculae to furcal radiolucency, restorable tooth having adequate crown structure for isolation using rubber dam.

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Table 1. Mean Reduction Of Microbial Count In Group A

Microflora	Sample	Mean CFU/ml	SD	SE	Mean difference (CFU/ml)	Z value	P value
Aerobic	Before Instrumentation	41.87x 10^3	82.93x 10^3	18.54x 10^3	40.1 x 10^3	3.921	<0.001*
	After Instrumentation	1.73x 10^3	2.94x 10^3	0.657x 10^3			
Anaerobic	Before Instrumentation	307.2x 10^3	1224x 10^3	273.7x 10^3	304.4 x 10^3	3.920	<0.001*
	After Instrumentation	2.82 x 10^3	3.38x 10^3	0.75x 10^3			

**p<0.001 (highly significant)

Exclusion Criteria: Presence of soft tissue abscess or sinus, internal and/ or external resorption in the roots, teeth with mechanical or carious perforation of pulpal floor, excessive pathologic bone loss involving the crypt of developing permanent successor and presence of a dentigerous or follicular cyst.

Diagnosis was made by a single investigator who also performed the clinical procedure. The parents and/or guardians were informed about the condition of the child's dentition. The nature of the study was briefly explained to them and written consent was obtained prior to the study. Following administration of local anesthesia, isolation with rubber dam was carried out. In order to eliminate micro-organisms from the operative field, the surface of both rubber dam and teeth were disinfected with 5% tincture of iodine.[9,10]

Access cavity was prepared using a #330 high speed bur with water spray, and entire roof of the pulp chamber was removed with #8 round bur, at slow speed. Necrotic tissue from the pulp chamber was removed using a sterile and sharp spoon excavator. After obtaining straight line access, pulp tissue was extirpated from the root canals using H files. Working length was then determined in each canal by measuring the length of the canal from mesiobuccal or distobuccal cusp to the apex of the root. The working length was kept 1mm short of the radiographic apex.[11]

For the purpose of instrumentation and sampling, the palatal canal of maxillary molars and the distal canal of mandibular molars were selected. Prior to sampling, Cavit™ (3M ESPE) was used to seal the orifices of both buccal canals of maxillary molars, and both mesial canals of mandibular molars. In this manner, contamination from other canals was prevented.[9]

An initial sample was taken from the root canal by placing a #15 sterile absorbent paper point up to the working length and allowing it to saturate for thirty seconds. It was then placed in a sterile vial containing 1ml of thioglycollate broth. (Hi media laboratories, Mumbai, India)

In all teeth, a glide path was established using a #15 k file and followed by a #20 K file. The canals were prepared together with intermittent irrigation using a standard volume of 5ml of normal saline. According to type of root canal instrumentation to be used for canal preparation, the sixty primary molars were systematically randomly assigned to three groups of 20 teeth each. Every first tooth was assigned to group A, every second tooth was assigned to group B and every third tooth was assigned to group C.

Instrumentation in each group was as follows: Group A: Rotary NiTi files (HERO Shaper- MicroMega, Geneva, Switzerland) were used with 1:64 Anthogyr gear reduction handpiece (Dentsply, Switzerland) at a speed of about 450 rpm.[5] The canals were first instrumented up to coronal one third using Endoflare (MicroMega, Geneva, Switzerland). Enlargement of canal up to the working length, was done using HERO shaper files, according to the sequence recommended by the manufacturer.

Group B and Group C: Instrumentation was carried out manually with hand nickel titanium K files (Dentsply, Switzerland) and hand stainless steel K files (Mani Inc, Japan), respectively. A file that showed resistance to intracanal placement, till the working length was selected as an initial file. The canals were enlarged up to three sizes more than that of the initial file, using 'pullback motion'.

Following complete instrumentation, a second sample was taken from the root canal in the manner described earlier. Cavit seal over the remaining canals was then removed. The remaining canals were also instrumented in a similar manner and final irrigation of all canals was carried out using 1% sodium hypochlorite. The canals were dried using sterile absorbent points and obturated with Endoflas FS (Sanlor and Cia. S.en., Cali, Colombia)

All the teeth were restored with Miracle mix (GC *Corp*, Tokyo, Japan) and a final restoration with stainless steel crown (3M ESPE) was given within one week of obturation.

Processing of microbial sample

The samples were transferred to the laboratory within two hours for microbial culture and quantitative analysis of micro-organisms. Each sample was serially diluted with normal saline by adding 1ml

Table 2. Mean Reduction Of Microbial Count In Group B

Microflora	Sample	Mean CFU/ml	SD	SE	Mean difference (CFU/ml)	Z value	P value
Aerobic	Before Instrumentation	820.5 x 10^3	2143.7 x 10^3	479 x 10^3	792.2 x 10^3	3.920	<0.001*
	After Instrumentation	28.3 x 10^3	111.3 x 10^3	24.88 x 10^3			
Anaerobic	Before Instrumentation	6487 x 10^3	19357 x 10^3	4328 x 10^3	6090.6 x 10^3	3.920	<0.001*
	After Instrumentation	396.4 x 10^3	1719 x 10^3	384 x 10^3			

**p<0.001 (highly significant)

Table 3. Mean Reduction Of Microbial Count In Group C

Microflora	Sample	Mean CFU/ml	SD	SE	Mean difference (CFU/ml)	Z value	p value
Aerobic	Before Instrumentation	49.27 x 10^3	99.00 x 10^3	22.14 x 10^3	45.97 x 10^3	3.920	<0.001*
	After Instrumentation	3.3 x 10^3	5.00 x 10^3	1.12 x 10^3			
Anaerobic	Before Instrumentation	51 x 10^3	1890.35 x 10^3	422.69 x 10^3	46.4 x 10^3	3.920	<0.001*
	After Instrumentation	4.6 x 10^3	173.76 x 10^3	38.85 x 10^3			

**p<0.001 (highly significant)

of the sample to 9 ml of normal saline. This was repeated thrice in order to obtain 10^{-4} dilution. From this dilution 1 ml each was inoculated by the spread plate method on to blood agar and thioglycollate agar plates (Hi media laboratories, Mumbai, India)

The blood agar plates were incubated aerobically at 37^0 C for three days. The thioglycollate agar plates were incubated at 37^0 C in an anaerobic jar containing 5% hydrogen, 10% nitrogen and 85% carbon dioxide for seven days. Total bacterial count was recorded by another examiner who was blind to the groups from which samples were obtained. A digital colony counter was used and number of colonies were expressed as CFU/ml.

Data obtained was subjected to statistical analysis using Wilcoxon signed rank test for pair-wise comparison of mean values; and one-way ANOVA to find out the significant difference between the three independent groups. Significance was predetermined at a p value of 0.05 or less. The bio- statistician was blind to the nature of study and the sampling method followed.

RESULTS

Following root canal instrumentation, there was significant reduction in the root canal microbial flora of the canals in all 3 groups. In group A, there was a 96% reduction in aerobic and 99% reduction in anaerobic microbial count. (Table 1) The percentage of reduction in group B was 97% and 94% for aerobic and anaerobic microbial count, respectively. (Table 2) In group C, there was a reduction of 91-93% % in both aerobic and anaerobic microbial count following root canal instrumentation. (Table 3). However on comparison of instrumentation, there was no significant difference between the groups in reduction of aerobic and anaerobic microflora. (Table 4)

DISCUSSION

It is well established that bacteria are the primary cause of endodontic infection and is poly-microbial in nature.[12-15] The success of endodontic treatment depends on many factors and the reduction or elimination of bacterial infection is the most important one. Due to the involvement of bacteria and clinical endodontics, the rationale for development of treatment protocols are based on data involving microbiological root canal sampling (MRS).[9,16-18] In other words, MRS is the very foundation of clinical endodontics.

Table 4. Comparison Of Reduction In Microbial Count Between The Three Groups

Groups	Mean Reduction in Aerobic microflora (CFU/ml)	Mean Reduction in Anaerobic microflora (CFU/ml)
Group A	40.1 x 103	304.4 x 103
Group B	792.2 x 103	6090.6 x 103
Group C	45.97 x 103	46.4x 103
F Value	2.614	1.923
P Value	0.082	0.156

In this study NiTi files (hand and rotary) were compared with the more commonly used conventional stainless steel hand files. The manual technique followed in canal preparation remained the same for both SS files and NiTi files, with metal composition and properties of the files being the difference. Further, hand NiTi files which have a similar composition with that of rotary files were compared using the manual and rotary instrumentation technique.

In primary teeth, it is preferable to use a low speed handpiece, with continuous torque and 150-300 rpm rotation.[4,5,20] In the present study an anthogyr handpiece, which is a low-speed constant torque (gear reduction of 1:64) hand piece was used. Due to its small head and simplicity, this hand piece was found to be more convenient for use in children.

The tortuous and irregular root canal walls of primary molars require to be minimally shaped in order to avoid perforation of canals.[4,5] Prior to the use of rotary files, the Endoflare was used to enlarge the coronal one-third of the canal. The Endoflare is a separate instrument that can be used in combination with files to aid instrumentation.[3] It is a #25 size instrument, with 0.12 taper, a blade length of 10 mm and it is used only to flare the coronal third at the beginning of shaping.[3]

Instrumentation in primary teeth must be performed with small diameter files of conic predefined form.[20] One of the newer rotary systems that incorporates this design together with high flexibility and constant taper is the HERO Shaper (Micro-Mega). HERO is the acronym for High Elasticity in Rotation (HERO).[3] They have improved features such as, a short handle for better access and blades with a non-cutting tip.[3]

The clockwise motion of the rotary files pulls pulpal tissue and dentin out of the canal as the files are engaged. The file was used in an 'in-and-out' (pecking) motion with light apical pressure. The "Archimedes screw" action may also pull debris and bacteria out of canals during instrumentation, resulting in cleaner canals.[9]

Mechanical instrumentation is an important contributor in elimination of bacteria and their by-products in infected root canals. Manual stainless steel and NiTi hand files are reported to successfully reduce intra canal flora by 100 to 1000 folds.[16] Rotary instrumentation has been shown to produce moderate to heavy smear layer on the canal walls.[21,22] Irrigation is necessary to suspend and rinse away debris created during instrumentation, to act as a lubricant for instruments, and to remove the smear layer that forms on instrumented dentine surfaces. Antimicrobial solutions should not be used

until after a microbial sample has been taken from the canal.[23] Saline is an inert root canal irrigant,[24,25] and has been reported not to have influence on the removal of smear layer.[23,26-28] Since the objective of the study was to evaluate the efficacy of instruments per se on root canal flora, instrumentation together with only saline irrigation was done. In this way, any bias resulting from the use of antimicrobial agents was excluded.

The type of culture medium used influences the results of root canal cultures. During culturing some cells may die. This depends on the nutritional demands of the bacteria, how the sample is protected from oxygen exposure and on the inoculation time. Hence thioglycollate agar and blood agar media were used for cultivation of anaerobic and aerobic micro-organisms, respectively.[29] Cultivation approaches makes it possible to identify a great variety of microbial species in a sample, including those that are not being sought after. [30]

Culturing allows all viable bacterial cells in a sample to grow which can be measured as colony-forming units. Some bacterial species have very stringent environmental and nutritional requirements that preclude culture on solid media.[31] Bacteria may be undetectable by culture if the number of cells is extremely low or bacteria are injured but not dead.[30] Molecular methods such as Polymerase Chain Reaction (PCR) are a highly sensitive assay for DNA detection; but cannot distinguish between viable and dead cells by their mononucleotide sequence. Thus, there may be an over estimation of canal flora.[32] Although PCR based methods are highly specific, precise and rapid they cannot detect un-identified or non-targeted species and cell numbers cannot be measured with conventional PCR.[31] Culturing has the advantage that contamination during laboratory manipulation may be more readily recognized than with molecular procedures. Thus isolation and cultivation of microorganisms remain essential tools for the analysis of the endodontic pathogens.[31]

The root canal flora is a combination of bacterial species, with the predominance of anaerobic bacteria, in root canal infections.[14,15,33,34] The number of bacterial species in an infected root canal may vary from one to more than 12, and the number of bacterial cells varies from $< 10^2$ to $> 10^8$ per sample.[31] In our study anaerobic bacterial counts were observed to be much higher in all the root canal samples. This was in accordance to a study on primary teeth with necrotic pulps which found anaerobic species in all root canals and 68.4% of root canal samples showed a poly-microbial nature.[34]

In the present study, significant bacterial reduction in canal flora was achieved with each type of canal instrumentation technique followed. This was similar to Dalton et al who also showed no significant difference between two instrumentation techniques used.[9] A recent *in vivo* study that used stainless steel hand files in mesiobuccal canals and NiTi instruments in mesio-lingual canals of the same lower molars showed no difference in their respective ability to eliminate residual intracanal infection after instrumentation.[35]

Our finding was similar to that of Bystrom and Sundqvist,[36] who reported more than 10 fold reduction in quantifiable growth of root canal microflora after instrumentation with saline irrigation. The control of bacteria within the root canal might appear to be straightforward since such a large proportion of the bacterial flora is sensitive to oxygen.[1] However, the penetration of oxygen into the canal during instrumentation does not seem to have any significant effect on the bacteria.[1] Hence in this study, the number of bacteria were significantly reduced, but neither instrumentation technique was able to render the canals completely free of bacteria. Microbiological root canal sampling is a passive sample of the main root canal space, which does not include inaccessible areas; such as accessory canals and dentinal tubules. In comparison to a single negative culture obtained in our study, Bystrom and Sundqvist were able to produce negative cultures in 53% of infected canals.[16] This difference could be due to instrumentation and saline irrigation repeated over five appointments in their study.

Further studies comparing the cleaning efficacy of different rotary systems and irrigation protocols in primary teeth can be carried out. Individual bacterial species in root canals of primary teeth could be identified. Rotary files were observed to be as effective as manual instruments in reducing root canal flora. Efficient cleaning of root canals with reduction in time spent on preparation is an important consideration in primary teeth and children. However, knowledge of rotary systems and experience is essential.

CONCLUSION

Rotary NiTi files were as efficient as conventional hand instruments in significantly reducing the root canal microflora. Conventional stainless steel files, NiTi files and rotary files were all effective in significantly reducing root canal flora. However, there was no significant difference between manual and rotary instrumentation techniques in microbial reduction of root canals.

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