Comparative evaluation of Apical Microleakage in root canals containing separated endodontic files: An in vitro study

ABSTRACT

Aims: The aim of the present in vitro study was to evaluate apical seal in the root canals containing separated instrument (Hand k files, Hyflex CM and Reciprocation Neoendo file) using dye penetration method.
Study design: An in vitro study
Place and Duration of Study: Department of Conservative Dentistry & Endodontics, SGRD Institute of Dental Sciences, Amritsar, Punjab, India between Feb 2022 and Nov 2023.
Methodology: In this study 3 different file systems were used for root canal preparation of 90 teeth. The files were intentionally separated (n=20 each) at the apical third and tooth was obturated using lateral condensation method along with 2 control groups (n=5 each) and the analysis of microleakage was done using dye penetration method under stereomicroscope.

Results: The highest mean microleakage was observed in Hyflex CM (5.49mm) group followed by Hand K file (3.338mm) whereas least microleakage was observed in samples containing separated Reciprocation file Neoendo (3.220mm).
Conclusion: Maximum microleakage was observed in Hyflex CM files and least in Reciprocation File Neoendo group.

Keywords: Continuous rotary motion; Dye penetration; Reciprocation; Microleakage; Apical seal

1. INTRODUCTION

In endodontic therapy, the root canal space is biomechanically prepared using endodontic instruments to remove microbial pathogens and create a surface free of debris. It also ensures adequate space for the irrigants to reach till the apical third and disinfect the canal space. But instrument fracture is an adverse sequelae of root canal shaping.

Although the mere existence of an instrument fragment within the tooth does not inherently impact the result of endodontic treatment, improper management of this condition can compromise the eradication of infected and/or vital tissues by endangering chemo-mechanical disinfection and obturation procedures [1].

According to Madarati et al. (2008)[2] various factors influencing instrument separation include operator proficiency, complicated root canal anatomy, overuse of the instrument, instrument metallurgy and inherent micro cracks, size and cross-section of file, sterilisation, calcified canals etc. and these factors should considered to determine overall prognosis of treatment.

Kosti et al. (2011)[3] discovered that maximum separation ocurred in canals with extreme curvature (60±10°). Also, the chances of breaking a file in the apical regions was thirty-three times higher than in the cervical region [4, 25, 26].

Once separated the instrument may be bypassed, or retrieval attempts may be made with retrieval kits, ultrasonics and the more recent minimally invasive gentle wave method but sometimes, it is impossible to retrieve the split piece. Therefore, endodontists want to include them in the final root canal filling.[5] Empirical data indicates that, adequately cleansed and sealed canals are not negatively affected by presence of fractured tools inside the tooth canal.[6]

So the present study seek to evaluate the effect of separated instruments with varying file designs and cross-section on the apical seal of the tooth and the microleakage was measured using dye penetration method. The null hypothesis under consideration was:

There will be no difference in microleakage values in root canals containing separated files as compared to canals without instrument separation.

2. MATERIAL AND METHODS

To assess the aftermath of a separated tool on the apical seal of obturated canals containing separated files, freshly removed teeth were collected from the Department of Oral and Maxillofacial Surgery and then evaluated for inclusion and exclusion criteria.

INCLUSION CRITERIA:

1. Permanent teeth with full root formation.

2. Restoration- and crack-free teeth.

EXCLUSION CRITERIA:

1. Teeth with fracture lines
2. Teeth with periodontal defects.

Procedure: Using an ultrasonic scaler, hard deposits were removed and organic debris was cleaned from the extracted teeth. The teeth were preserved in 10% formalin until needed, after being cleaned with distilled water to get rid of any soft tissue fragments.

The selected teeth were prepared using either of the three endodontic files as allocated by Random allocation software 2.0.

Biomechanical preparation**:** After determining the working length using Ingle's method, three separate file systems were used to prepare these canals:

2.1 Reciprocation file Neo-Endo: The glide path was established by 17/4% file(NeoEndo flex) and root canal preparation was done by 20 no. file (Reciprocation File NeoEndo). A 30-gauge side-vented needle was used to irrigate the root canals during preparation. First, 5.25% sodium hypochlorite solution was used, followed by 17% ethylenediamine tetra acetic acid (EDTA). Normal saline was used as the last irrigant.

2.2 Hand Files:,The 15/.02 hand K file (DentsplyMaillefer) was used to establish glide path. Root canal preparation was carried out by DentsplyMaillefer's 20/.02 K-file. A 30-gauge side-vented needle was used to irrigate the root canals during preparation. First, 5.25% sodium hypochlorite solution was used, followed by 17% EDTA. The final irrigant was normal saline.

2.3 HyFlex CM (coltene): The glide path was established by 15/.04 file(HyFlexCM) and root canal preparation was done by 20/.04 file(HyFlex CM). A 30-gauge side-vented needle was used to irrigate the root canals during preparation. First, 5.25% sodium hypochlorite solution was used, followed by 17% EDTA. The final irrigant was normal saline.

After which the endodontic file was split by nicking it in the apical third of the canal. Based on the endodontic file fractured, three groups of sixty teeth (experimental samples) were created.

GROUP-A:-Reciprocation File Neo Endo(Orikam Healthcare India Private Limited)

GROUP- B:-Hand K-files (DentsplyMaillefer)

GROUP- C:-HyFlex CM (ColteneWhaledent, Altstetten, Switzerland)

All the canals were then obturated using Cold Lateral Compaction Technique above the divided file: The canal was first dried and the apical length of the guttapercha was trimmed in accordance with the length of the separated file, and the master guttapercha cone was chosen based on the most recent file used at working length. Sealer was coated on canal walls and the modified guttapercha cone. In order to condense the apical portion of the canal, a spreader was placed alongside the main cone. Similarly, secondary guttapercha cones were used until the canal was completely filled.

5 positive and negative samples were prepared for each group that constituted of biomechanically prepared root canals without obturation and root canals obturated with lateral compaction technique after biomechanical preparation respectively. The produced samples were kept in a 100% humidity environment at 37ºC.

Using a stereomicroscope and the dye penetration method, microleakage was evaluated: With the exception of the apical 2-3 mm, samples were covered in nail varnish. After the varnish coat had dried, it was submerged in dye for 72 hours in various specimen containers. After rinsing the samples under running water, a surgical blade was used to remove the nail polish. Using a diamond disc and straight handpiece, all specimens were longitudinally sectioned in the buccolingual direction. The depth of dye penetration was measured in millimetres off all the samples using an Image ruler (Version 1.1). Representative images of all the groups are depicted in Fig. 1. After calculation, the data was analysed using One-way ANOVA and Post-hoc Bonferroni Analysis of Variance

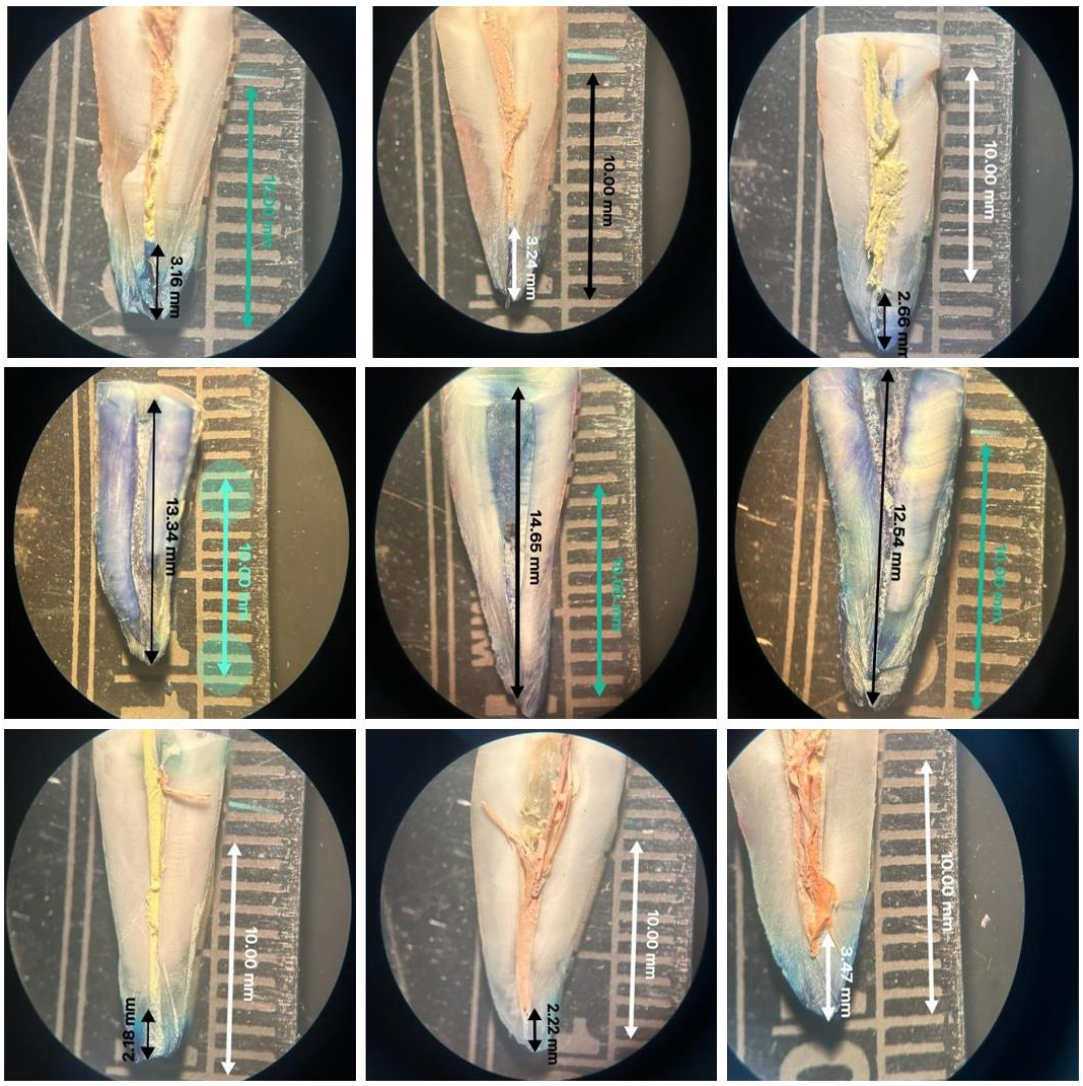

Fig 1: Dye penetration in samples containing separated instrument : Experimental samples (A-C) : Fig.A – Reciprocation Neoendo file, Fig. B- Hand k file, Fig. C- Hyflex CM. Positive control samples(D-F): Fig.D – Reciprocation Neoendo file, Fig.

E- Hand k file, Fig. F Hyflex CM and Negative control samples (G-I): Fig.G – Reciprocation Neoendo file, Fig. H- Hand k file, Fig. I- Hyflex CM

Table 1: Mean Microleakage among different experimental groups

Groups	N	Mean
Group-A: Neo Endo Reciprocation Group	20	3.220
Group-B: Hand K File Group	20	3.338
Group-C: Hyflex CM File Group	20	5.498
P -value	0.003	

Applied test : One-way ANOVA

Significant (*P* <0.05)

Table-2: Multiple comparisons between different study groups:

Groups comparisons	Mean Difference (I-J)	Sig.
Group-A & B	-0.118	1.000
Group-A & C	-2.27800*	0.007
Group-B & C	-2.16000*	0.011

*. The mean difference is significant at the 0.05 level.

Test applied: Post-hoc Bonferroni Analysis of Variance

Table 3: Comparison between experimental and control values of microleakage within various groups:

		Group-A		Group-B		Group-C	
Particulars	N	Mean	±SD	Mean	±SD	±SD	SE
Experimental Group	20	3.220^{a}	0.833	3.338^{a}	1.347	3.58	0.801
Negative control	5	3.178^{a}	1.083	3.162^{a}	1.021	0.935	0.418
Positive control	5	13.600^{b}	1.181	11.468^{b}	2.325	3.135	1.402
P –value		< 0.001		< 0.001		0.003	

Applied test: One-way ANOVA

The groups with same letters; $P > 0.05$ i.e. have insignificant variation & different letter in the superscripts differ significantly ($P<0.05$)

3.RESULTS AND DISCUSSION

3.1 Results: The highest mean microleakage values were seen in Group- C (5.498mm) followed by Group- B (3.338mm) and Group-A(3.220mm) in experimental samples containing fractured instruments and the difference was significant(*P*=.03) as depicted in Table-1. The intergroup analysis revealed statistically significant difference between group-A & C (*P*=.007) whereas the dissimilarity between Group-A & Group-B (*P*=1.00) and Group-A & Group-C (*P*=1.00) was insignificant as displayed in Table-1.

Table-3 depicts an intra-group comparison between experimental, positive and negative samples of various groups. The Group-A revealed that the difference between microleakage values in experimental and negative control group was insignificant whereas that of experimental and positive control was significant (*P*<0.05).

3.2 Discussion: Endodontics is the branch of science which deals with the identification, prevention and treatment of pathalogical pulpal, periapical and periradicular conditions [7]. The cleaning and shaping of root canals is crucial for endodontic therapy to be successful as it decreases the microbial load and achieve a complete 3-D seal but is assosciated with complication of file separation. This is influenced by various factors like instrument cross-section, material of the file, kinematics etc.

Although mere existence of fragment may not complicate the prognosis of tooth but it might hinder in the disinfection of canal. So in the current investigation the effect of instrument separation of endodontic files with varying cross-section and

operated using different operation motions (Reciprocating, manual instrumentation and continuous rotary motion) on the microleakage of samples was evaluated.

The current investigation revealed significantly higher (*P*=0.003) microleakage values in Group-C followed by Group-B & A respectively. The reason for lower microleakage values may be attributed to the S-shaped cross-section of Reciprocation Neo endo files which allowed better removal of the dentinal debris. In contrast to Hand K files possessing rectangular cross-section and Hyflex CM files- a three cornered cross-section which had relatively smaller gaps for debris clearing and encouraged smear layer collection. Also, the S-shaped cross-section allowed sealer to flow along the broken file providing better obturation. These observations corroborate with the findings of various authors [8-11].

The inter-group comparison amongst Group-A & B disclosed insignificant difference (*P* =1.00) between the two groups and corroborates with observations of Subramaniam et al. (2016) [12] who compared smear layer formation between rotary and hand files and concluded that both performed similarly with statistically insignificant difference (p>0.05). These findings may be attributed to increased accumulation of smear layer, dentinal chips and lower clearance of produced debris in Hyflex CM files as compared to group-A Neoendo reciprocation files with S-shaped cross-section that ensured efficient elimination of the debris due to the large gaps between the cutting blades and hence eliminating any obstruction to the smear layer removal.

On the contrary, dissimilarity between Group-A&C (p=0.007) and Group-B&C (*P* =0.011) was statistically significant. The higher microleakage in Hyflex CM files with a three-cornered cross-section might be due to increased accumulation of smear layer, dentinal chips and lower clearance of the produced debris as compared to group-A Neoendo reciprocation files. The superior performance of Hand k files may be attributed to the lower production of smear layer and debris in hand files as compared to the rotary endodontic files .The rotary files undergo more number of cycles leading to higher and thicker smear layer formation. As a physical barrier, the smear layer inhibited the sealer's adhesion and penetration into the tubules, which caused spaces to form between the dentinal walls and the obturation materials and increased micro leakage. These finding are in accordance to the observations of various authors [10, 13-17].

The intra-group comparison as depicted in table-3 revealed that the lowest microleakage values were seen in negative control samples of all the groups followed by the experimental samples and the difference between the two was insignificant (*P* >0.05).These results confer with findings of various authors [18-22] that reported higher microleakage values in obturated teeth obturated containing a separated file as compared to teeth without them. Similarly, in a study conducted Ok E and Ertas H (2014)[18] it was concluded that regardless of type of file , fracture of instrument will have a negative impact on the apical seal.

On the contrary, significantly higher (p<0.05) microleakage values were observed in positive control samples. This may be because positive control samples constituted biomechanically prepared root canals without obturation and the higher microleakage values are due to the absence of obturation material allowing higher amount of dye to penetrate and is in corroboration with findings of various authors [23, 24].

Thus the current investigation helps in analysing the effect on the fractured file on the apical seal and its overall impact on the prognosis of the endodontic therapy.

In order to guarantee the separation of endodontic file at the apical portion of tooth for the purpose of evaluating microleakage, the files were nicked at a distance of 3 mm. As a result, the separated fragment may not be as tightly bound to the dentin, with a decreased smear layer and debris formation, than the instrument separated due to torsional or cyclic fatigue in the canal, which could have resulted in higher microleakage values.

4. CONCLUSION

Within the shortcomings of this research The Reciprocation File Neoendo displayed the lowest microleakage values among the various study groups. Hence the present study validates the better performance of S-shaped reciprocation file systems and encourages their application as along with their superior fatigue resistance they also have least negative impact if left within the canal.

AUTHORS' CONTRIBUTIONS

'Author A' played major role in study design, establishing protocol, data collection and analysis and drafteding the manuscript. 'Authors B and C' also contributed in designing study, reviewing and analyzing data. 'Authors C and D'

conducted literature searches, data analysis and reviewed the final draft. All authors have read and approved the final version of the manuscript."

ETHICAL APPROVAL

The necessary ethical approval was obtained by the authors from Institutional ethical committee of Sri Guru Ram Das Institute of dental sciences and research, Amritsar.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include list the name, version, model, and source of the generative AI technology and as well as the all input prompts provided to a generative AI technology

Details of the AI usage are given below:

1.

2.

3.

REFERENCES

1. Zanza A, Reda R, Testarelli L. Endodontic Orthograde Retreatments: Challenges and Solutions. Clinical, Cosmetic and Investigational Dentistry. 2023; 31:245-65.
2. Madarati AA, Watts DC, Qualtrough AJ. Factors contributing to the separation of endodontic files. Br. Dent. J 2008; 204:241-5.
3. Kosti E, Zinelis S, Molyvdas I, Lambrianidis T. Effect of root canal curvature on the failure incidence of ProFile rotary Ni–Ti endodontic instruments. Int. Endod. J. 2011; 44:917-25.
4. Iqbal MK, Kohli MR, Kim JS. A retrospective clinical study of incidence of root canal instrument separation in an endodontics graduate program: a PennEndo database study J. Endod. 2006; 32:1048-52.
5. Sharifi R, Heidarzadi E, Azizi E, Khademi A, Khazaei S, Iranmanesh P. Effect of different separated nickel–titanium rotary instruments on apical microleakage: An: in vitro: study. Saudi Endod. J. 2022; 12:61-6. *DOI:* 10.4103/sej.sej_42_21
6. Godiny M, Hatam R, Khavid A, Khanlari S. Apical microleakage in root canals containing broken rotary instruments. Iran. Endod. J. 2017; 12:360-5. Doi: 10.22037/iej.v12i3.16656
7. Bergmans L, Van Cleynenbreugel J, Wevers M, Lambrechts P. Mechanical root canal preparation with NiTi rotary instruments: rationale, performance and safety. Am J Dent. 2001; 14(5):324-33.
8. Bürklein S, Hinschitza K, Dammaschke T, Schäfer E. Shaping ability and cleaning effectiveness of two single-file systems in severely curved root canals of extracted teeth: Reciproc and WaveOne versus Mtwo and ProTaper. Int. Endod. J. 2012; 45:449-61.
9. Chugh VK, Patnana AK, Chugh A, Kumar P, Wadhwa P, Singh S. Clinical differences of hand and rotary instrumentations during biomechanical preparation in primary teeth—A systematic review and meta-analysis Int. J. Paediatr. Dent. 2021; 31:131-42.
10. Shahraki HA, Saberi EA, Maserrat V, Dalaei M, Nejad SM. In Vitro Comparison of Apical Micro Leakage in Root Canal Prepared Wave One and Reciproc Files. Journal of Biochemical Technology. 2020; 1:65-70.

11. Zare Jahromi M, Zamiran S. Experimental study of smear layer and debris remaining following the use of four root canal preparation systems using scanning electron microscopy. Journal of Iranian Dental Association. 2013; 25:235-41.
12. Subramaniam P, Girish Babu KL, Tabrez TA. Effectiveness of rotary endodontic instruments on smear layer removal in root canals of primary teeth: A scanning electron microscopy study. Journal of Clinical Pediatric Dentistry. 2016;40(2):141-6.
13. Maheswari D, Mallick RR, Shandilya A, Solanki H, Panda S, Sarangi P. Quantitative evaluation of apically extruded debris during biomechanical preparation using hand K-file, protaper next, and waveone–An In Vitro study. J. Pharm**.** Bioallied Sci. 2022; 14:S802-7.
14. Peralta-Mamani M, Rios D, Duarte MA, Santiago Junior JF, Honorio HM. Manual vs. rotary instrumentation in endodontic treatment of permanent teeth: A systematic review and meta-analysis Am. J. Dent. 2019; 32:311-24.
15. Khademi A, Saatchi M, Shokouhi MM, Baghaei B. Scanning electron microscopic evaluation of residual smear layer following preparation of curved root canals using hand instrumentation or two engine-driven systems. Iran. Endod. J. 2015; 10:236-9.
16. Schäfer E, Schulz-Bongert U, Tulus G. Comparison of hand stainless steel and nickel titanium rotary instrumentation: a clinical study. J. Endod. 2004 Jun 1; 30(6):432-5.
17. Ali M, El Ashry S, Ismail AG. Comparative evaluation of smear layer Formation of WaveOne Gold, Protaper Gold and manual files: An in vitro SEM study. Curr Sci Int. 2020; 9:381-6. DOI: 10.36632/csi/2020.9.2.33
18. Evren OK, Ertas H, Iysal B. Effect of separated rotary instruments on the apical microleakage of root canal fillings. Atatürk Üniversitesi Diş Hekimliği Fakültesi Dergisi. 2014; 24:246-51.
19. Sharifi R, Heidarzadi E, Azizi E, Khademi A, Khazaei S, Iranmanesh P. Effect of different separated nickel–titanium rotary instruments on apical microleakage: An: in vitro: study. Saudi Endod. J. 2022; 12:61-6. *DOI:* 10.4103/sej.sej_42_21
20. Taneja S, Chadha R, Gupta R, Gupta A. Comparative evaluation of sealing properties of different obturation systems placed over apically fractured rotary NiTi files. J. Conserv. Dent. 2012; 15:36-40.
21. Hegde J, Bashetty K, Kumar KK, Chikkamallaiah C. Comparative evaluation of the sealing ability of different obturation systems used over apically separated rotary nickel-titanium files: An: in vitro: study. J. Conserv. Dent. 2013; 16:408-12.
22. El-Wahed A, Zaazou AM, El Mallakh BF. Apical Sealing Efficiency of Different Obturation Techniques over Apically Broken Rotary Nickel-Titanium Files (An in Vitro Study). Alexandria Dental Journal. 2015 Jul 1; 40:46-50.
23. Mashalkar S, Selvakumar G, Diwanji P, Indi S, Warad J. Apical microleakage in root canal-treated teeth containing broken hand files obturated with two different obturating materials: An: in vitro: study. Endodontology. 2019; 31:68-71. *DOI:* 10.4103/endo.endo_77_18
24. Saunders JL, Eleazer PD, Zhang P, Michalek S. Effect of a separated instrument on bacterial penetration of obturated root canals. J. Endod. 2004; 30(3):177-9.
25. Magura ME, Kafrawy AH, Brown CE, Newton CW. Human saliva coronal microleakage in obturated root canals: an in vitro study. Journal of Endodontics. 1991 Jul 1;17(7):324-31.
26. Von Fraunhofer JA, Fagundes DK, McDonald NJ, Dumsha TC. The effect of root canal preparation on microleakage within endodontically treated teeth: an in vitro study. International Endodontic Journal. 2000 Jul;33(4):355-60.