Original Article

DOI: 10.7860/JCDR/2026/82176.22223

Dentistry Section

Comparison of Root Fractures Associated with Three Different Endodontic Irrigants of Varied pH: An In-vitro Study

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ABSTRACT

Introduction: Vertical root fractures present significant challenges, and most often, the affected tooth is indicated for extraction. Root canal irrigants are employed synergistically alongside filing instruments during root canal therapy. The acidic nature of such irrigants is responsible for the removal of dentin during the process of biomechanical preparation. This subsequently leads to weakening of the tooth structure, ultimately leading to the fracture of the tooth.

Aim: To compare the incidence of incomplete root fractures after treatment with three different root canal irrigants- citric acid, Ethylenediaminetetraacetic acid (EDTA), Regulated Ethylenediaminetetraacetic Acid (R-EDTA at two different Potential of Hydrogen (pH) levels (5.5 and 10.5).

Materials and Methods: This in-vitro research was undertaken at Manav Rachna Dental College, Faridabad, Haryana, India for a period of six months from July 2024 to December 2024. Total of 48 human mandibular lateral incisors were selected and split into six groups on the premise of application of endodontic irrigants- citric acid, EDTA, R-EDTA with a particular pH. The irrigants employed were 17% citric acid pH 10.5 (Group 1), 17% citric acid pH 5.5 (Group 2), 17% R-EDTA pH 10.5 (Group 3), 17% R-EDTA pH 5.5 (Group 4),17% EDTA pH 10.5 (Group 5) and 17% EDTA pH 5.5 (Group 6). The samples were then subjected to Oil Red O Stain and analysed under a microscope for fractures. The data obtained was subjected to a Chi-squared test.

Results: While no significant statistical differences were found between treatment groups (p-value>0.05), a trend was observed where lower pH formulations demonstrated more fractures compared to higher pH formulations of the same irrigant. The group treated with 17% REDTA at pH 5.5 showed the highest number of tooth fractures.

Conclusion: Lowering of pH might have a role in causation of incomplete root fractures. Further analysis is required to assess the impact of R-EDTA on the structural integrity of teeth.

Keywords: Chemomechanical preparation, Citric acid, Oil red O stain

INTRODUCTION

Chemomechanical preparation, an integral part of root canal treatment, often generates substantial amounts of debris and smear layer [1,2]. Ethylenediaminetetraacetic Acid (EDTA) is commonly employed for eradicating the inorganic part of the smear layer. It helps in reducing friction in endodontic instruments and enhances adhesion of materials to the walls of the root [3-5]. Citric acid combines with metals, resulting in chelates [6]. It has also been used for conditioning of dentin, and it is chemically stable [7,8]. The Regulated Ethylenediaminetetraacetic Acid (R-EDTA) is another irrigant introduced by Kennedy with a composition of EDTA, Sodium Hypochlorite (NaOH), Cetyltrimethylammonium Bromide (CTAB) and Distilled Water (DW) [9]. The vertical root fracture is a linear crack of the root, expanding throughout the whole width of dentin right from the canal wall till the periodontium [10]. Vertical root fracture is an issue of great clinical concern as it typically results in a poor prognosis for the tooth. Although in certain conditions a cracked tooth can be salvaged with a hemisection, however, extraction of the affected tooth is the ultimate solution [11]. Vertical root fractures are often linked to the substantial occlusal load on the teeth. They may also occur as a result of undue pressure from operative drills carried out during root canal treatment. Loss of tooth structure during instrumentation and obturation can weaken endodontically treated teeth, making them susceptible to fracture [12]. Endodontic irrigants have an impact on endodontically treated teeth to fracture. Studies conducted in the past have reinforced the perception that endodontic irrigants make a tooth frail, leading it to fracture [12-14]. The impact of irrigants on dentin removal has been assessed in the past, but not many studies have taken into account the effect of irrigants at two different levels of acidity. Thus, this research aimed to compare the incidence of incomplete root fractures after manipulation with the three above-mentioned endodontic irrigants at pH levels of 10.5 and 5.5, respectively.

MATERIALS AND METHODS

This in-vitro research was undertaken at Manav Rachna Dental College, Faridabad, Haryana, India for a period of six months from July 2024 to December 2024. A total of 48 fully developed extracted human mandibular lateral incisor teeth with straight and one canal were chosen and examined radiographically using an RVG 5200 (Kodak Dental Systems, Atlanta, GA, USA) from both labiolingual and mesiodistal perspectives to verify the existence of only one canal and assess its morphology.

Inclusion criteria: It consisted of teeth that were extracted due to weak periodontal support with fully developed root apices with single and straight root canals.

Exclusion criteria: Teeth were excluded if, under an operating microscope, teeth were found to have fissures, cracks, calcification, or caries affecting the root. In addition, teeth with developmental anomalies were also not included in the study. Before the experiment, the teeth were kept in a formalin container, which served as the storage medium.

Sample size calculation: The sample size was estimated by convenience sampling. The 48 mandibular lateral incisors were arbitrarily split into 6 sets of 8 teeth each.

Study Procedure

The crown portions of the samples were resected with a Z high-speed bur. The 10-K file was pushed into the major diameter, and 0.5 mm

was removed from the total to estimate the working length. The root canals were manipulated with an F1 ProTaper instrument. After preparation, specimens were allotted into six groups of eight teeth each [Table/Fig-1] and irrigation of the root canals was done with normal saline and the last and final irrigation was done with 17% citric acid pH 10.5 (Group 1), 17% citric acid pH 5.5 (Group 2), 17% R-EDTA pH 10.5 (Group 3), 17% R-EDTA pH 5.5 (Group 4),17% EDTA pH 10.5 (Group 5) and 17% EDTA pH 5.5 (Group 6) respectively. The two different levels of pH of irrigants were kept to determine their impact at basic (10.5) and acidic (5.5) levels.

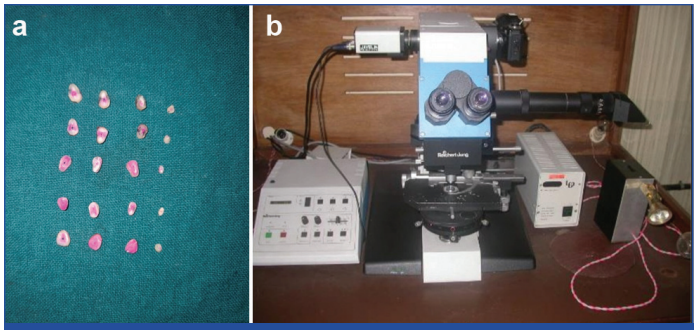
[Table/Fig-1]: Tooth samples- Tooth specimens were allotted into six groups of eight teeth each.

Prepared samples: The endodontic irrigants were grouped into two similar amounts for each concentration, and pH was calibrated to 10.5 and 5.5 by adding an alkali (sodium hydroxide) and verified with pH indicator strips.

Manual dynamic agitation was used to amplify the penetration of irrigants inside the root. Thereafter, the canals were dehydrated with paper points. No obturation was performed.

The teeth were placed in storage for 1 week in 10% buffered formalin before staining with 5% Oil Red O. Thorough rinsing of specimens was done with water after staining. The rinsing of the specimens with water was done to differentiate the stained fractures caused due to irrigation and instrumentation from the fractures which can be caused due to subsequent sectioning [11]. Each specimen was poured into a resin and later sectioned into 4 sections, and each section was examined under a Polyvar-2 microscope (Depew, New York, USA) attached with a macromagnifier [Table/Fig-2a,b].

A tooth was classified as fractured if at least one histological section demonstrated either an incomplete or complete fracture. Complete root fractures are defined as fractures that expand all through the root canal to the external side of the root, while incomplete root fractures are said to be the ones which do not reach the root's external surface [14].



[Table/Fig-2]: a) Tooth sectioning, b) Polyvar-2 microscope.

STATISTICAL ANALYSIS

The data were inspected using the Statistical Package for the Social Sciences (SPSS), version 23.0 (Chicago, IL, USA). Tabulation of descriptive statistics was done. Chi-square test was applied. The level of significance was set at p-value< 0.05

RESULTS

The intergroup comparison based on the number of sections fractured showed no statistically significant difference among the six groups (p-value = 0.494). Although group 4 had the highest number of fractured sections 7 (21.9%), and group 1 had the lowest 2 (6.3%), these variations were not statistically significant. Overall, the majority of specimens across all groups remained unfractured 169 (88%), indicating that the experimental conditions or interventions did not cause notable differences in fracture incidence among groups [Table/Fig-3].

The intergroup comparison based on the number of teeth fractured revealed no statistically significant difference among the six groups (p-value=0.931). Although group 4 exhibited a relatively higher percentage of fractured teeth 3 (37.5%) compared to other groups, this variation was not statistically significant. Overall, 36 (75%) of teeth across all groups remained unfractured, indicating that the type of intervention or condition evaluated did not substantially influence the incidence of tooth fracture among the groups [Table/Fig-4].

DISCUSSION

The present study investigated the potential relationship between root canal irrigant pH and the incidence of incomplete root fractures in endodontically-treated teeth. While no statistically significant differences were observed between treatment groups, the findings reveal important trends that warrant further consideration.

The fractures that were observed in this study mainly comprised those that originated from the lumen of the root canal, those that were present tangentially and those that appeared wholly inside the dentin. This suggests that the fractures likely originated from the internal surface of the root canal wall, where the irrigant-dentin interaction is most intense. The use of Oil Red O staining to differentiate between treatment-induced fractures and those

Parameters	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)	Group 5 n (%)	Group 6 n (%)	Total N=48	p-value
Not Fractured	30 (93.8%)	28 (87.5%)	29 (90.6%)	25 (78.1%)	29 (90.6%)	28 (87.5%)	169 (88.0%)	0.494
Fractured	2 (6.3%)	4 (12.5%)	3 (9.4%)	7 (21.9%)	3 (9.4%)	4 (12.5%)	23 (12%)	
Total count	32 (100%)	32 (100%)	32 (100%)	32 (100%)	32 (100%)	32 (100%)	192 (100%)	

[Table/Fig-3]: Intergroup comparison based on the number of sections fractured.

Parameters	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)	Group 5 n (%)	Group 6 n (%)	Total N=48	p-value
Not Fractured	7 (87.5 %)	6 (75.0%)	6 (75.0 %)	5 (62.5 %)	6 (75.0%)	6 (75.0 %)	36 (75.0%)	0.931
Fractured	1 (12.5 %)	2 (25.0%)	2 (25.0 %)	3 (37.5 %)	2 (25.0%)	2 (25.0 %)	12 (25 %)	
Total count	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	48 (100%)	

[Table/Fig-4]: Intergroup comparison based on the number of teeth fractured.

resulting from specimen processing represents a methodological strength, ensuring that observed fractures were genuinely related to the irrigant effects rather than procedural artefacts. The fracture lines were usually positioned tangentially [Table/Fig-5] to the root canal rather than extending outwards [Table/Fig-6], which is consistent with the study by Holcomb J and Pitts D, which stated that there was no correlation between the location of appearance of fracture lines and the position of filing instruments in the root canal [15].

In the present study, lateral canals were readily differentiated with fracture lines, as the teeth selected were extracted using minimal pressure to prevent any iatrogenic damage. The 5% Oil Red O stain was employed in the current study because it penetrates dentin effectively, thereby making fracture lines more distinguishable under microscopic examination [16].

Intergroup comparison was done both section-wise and tooth-wise. The results displayed that no group exhibited statistically significant differences when compared to each other (p-value>0.05). However, the section-wise comparison revealed that lowering the pH of the respective irrigant did increase the number of fractured sections. This trend was observed across all three irrigants: citric acid, EDTA and R-EDTA.

This phenomenon can be explained by the fact that the pH significantly influences calcium availability through multiple mechanisms. The chelating efficacy of EDTA at elevated pH is high due to a decreased ratio of non ionised to ionised particles in the solution. This can be attributed to the fact that at higher pH, excess hydroxyl ions will interfere with the dissolution of hydroxyapatite, thereby reducing the availability of calcium ions for chelation. Conversely, at a lower pH, increased hydrogen ion concentration facilitates hydroxyapatite breakdown and enhances calcium accessibility for chelation [17].

The lowest number of fractured sections and teeth was observed in the pH 10.5 citric acid group. This is in coherence with Scelza MF et al., who examined the impact of citric acid on smear layer eradication and demonstrated that citric acid did not erode dentin and therefore did not contribute to dentin weakening [18]. Chockattu SJ et al., showed that when citric acid came in contact with calcium hydroxide, its pH decreased, which subsequently reduced its efficacy in removing calcium hydroxide [19]. It has been observed that citric acid effectively removes the smear layer without having any detrimental effects on root dentin [20]. Studies have shown that both citric acid and EDTA are similarly efficacious in erasing smear layer [21,22], but citric acid is more compatible and fit for use as compared to EDTA [18]. EDTA is a highly favoured root canal irrigant for eliminating the smear layer on the dentin [23]. When EDTA is used in combination with sodium hypochlorite, it dissolves the apatite in dentin, where the collagen matrix has already been degraded due to the action of the latter. This synergistic effect leads to the further weakening of the tooth structure [22].

A study by Dogan Buzoglu H et al., showed that EDTA reduces the wettability of the dentin. Therefore, short exposure times are considered less detrimental to the mechanical strength of teeth. Hence, for this reason, it is specifically recommended to use EDTA for less than two minutes, especially when used at higher concentrations [24]. Dotto L et al., in their study implied that administering EDTA as a final rinse after biomechanical preparation allows clinicians to gain its benefits in smear layer removal while minimising its adverse effect on dentin strength [25].

Another noteworthy finding was that pH 5.5 R-EDTA was associated with a higher incidence of section fractures, although the difference was not statistically significant compared to other groups. This may be attributed to the reduced surface tension resulting from the inclusion of a surfactant in the irrigant, which enhances its chelating efficacy [26].

Limitation(s)

The study was performed under laboratory conditions, which may not fully replicate the complex oral environment (saliva, temperature fluctuations, masticatory forces, bacterial biofilms). The effects of irrigants on dentin were assessed immediately after treatment; long-term effects on dentin strength and fracture resistance were not evaluated. The study was restricted to a relatively small sample size and a single type of dentin, without accounting for variations in tooth age, location, or structural differences, which may reduce the generalisability of the findings. Future studies should include larger sample sizes, varied irrigant concentrations, and longer evaluation periods under simulated clinical conditions. In-vivo studies are needed to validate the long-term effects of irrigants on dentin integrity and tooth survival.

CONCLUSION(S)

The present study demonstrated that irrigating solutions of varied pH can induce dentinal microcracks, potentially compromising tooth integrity over time. While no significant differences were observed among the irrigants in causing incomplete root fractures, citric acid appeared the least detrimental, followed by EDTA, whereas R-EDTA showed greater potential for dentin damage. Further research is warranted to explore the role of irrigant acidity and to develop safer formulations with minimal adverse effects on tooth structure.

REFERENCES

[1] Zehnder M. Root canal irrigants. J Endod 2006;32:389-98.
[2] Gutiérrez JH, Jofré A, Villena F. Scanning electron microscope study on the action of endodontic irrigants on bacteria invading the dentinal tubules. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1990;69:491-501.
[3] Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: Mode of action and indications for their use. Int Endod J. 2003;36:810-30.
[4] Dartar Oztan M, Akman AA, Zaimoglu L, Bilgiç S. Corrosion rates of stainless-steel files in different irrigating solutions. Int Endod J. 2002;35:655-59.
[5] Rotstein I, Karawani M, Sahar-Helft S, Mor C, Steinberg D. Effect of sodium hypochlorite and EDTA on mercury released from amalgam. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;92:556-60.
[6] Yamaguchi M, Yoshida K, Suzuki R, Nakamura H. Root canal irrigation with citric acid solution. J Endod. 1996;22:27-29.
[7] Salama FS, Abdelmegid FY. Six per cent citric acid is better than hydrogen peroxide in removing smear layer: An in vitro pilot study. Pediatr Dent. 1994;16:424-26.
[8] Di Lenarda R, Cadenaro M, Sbaizero O. Effectiveness of 1 mol L-1 citric acid and 15% EDTA irrigation on smear layer removal. Int Endod J. 2000;33:46-52.
[9] Kucuk M, Tunca YM, Erdem O, Cetinkaya S, Demirkaya K. Efficacy of preheated chelating agents on calcium ion removal from instrumented root canals. J Clin Exp Dent. 2021;13:1015-20.
[10] Lertchirakarn V, Palamara JEA, Messer.H.H . Load and strain during lateral condensation and vertical root fracture. J Endod. 1999;25:99-104.
[11] Onnink PA, Davis RD, Wayman BE. An in vitro comparison of incomplete root fractures associated with three obturation techniques. J Endod. 1994;20:32-37.
[12] Lam PP, Palamara JE, Messer HH. Fracture strength of tooth roots following canal preparation by hand and rotary instrumentation. J Endod. 2005;31:529-32.
[13] Uzunoglu E, Aktemur S, Uyanik MO, Durmaz V, Nagas E. Effect of ethylenediaminetetraacetic acid on root fracture with respect to concentration at different time exposures. J Endod. 2012;38:1110-13.
[14] Lindauer P, Campbell A. Vertical root fractures in curved roots under simulated clinical conditions. J Endod. 1989;15:345-49.
[15] Holcomb J, Pitts D. Further investigation of spreader loads required to cause vertical root fracture during lateral condensation. J Endod. 1987;13:277-84.
[16] Junbao Du, Li Zhaoa, Quan Kang, Yun He, Yang Bi. An optimised method for Oil Red O staining with the salicylic acid ethanol solution. Adipocyte 2023;12(1):2179334.
[17] Cury JA, Bragotto C, Valdrighi L. The demineralising efficiency of EDTA solutions on dentin. Oral surg Oral med Oral Pathol. 1981;52:446-48.
[18] Scelza MF, Pierro V, Scelza P, Pereira M. Effect of three different time periods of irrigation with EDTA-T, EDTA, and citric acid on smear layer removal. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;98:499-503.
[19] Chockattu SJ, Deepak BS, Goud KM. Comparison of the efficiency of ethylenediaminetetraacetic acid, citric acid, and etidronate in the removal of calcium hydroxide intracanal medicament using scanning electron microscopic analysis.An in-vitro study. J Conserv Dent. 2017;20:06-11.
[20] Hakan A, Cagatay B, Ertugrul K, Huseyin ST, Kubra YY, Ibrahim E, et al. Effect of citric acid irrigation on the fracture resistance of endodontically treated roots. Eur J Dent. 2014;8:74-78.

[21] Kaushal R, Bansal R, Malhan SA. Comparative evaluation of smear layer removal by using ethylenediamine tetraacetic acid, citric acid, and maleic acid as root canal irrigants: An in vitro scanning electron microscopic study. J Conserv Dent. 2020;23:71-78.
[22] Haiping X, Zhou Y, Zhang A, Lin F, Fu J, Fok ASL. Effects of concentrations of sodium hypochlorite as an endodontic irrigant on the mechanical and structural properties of root dentine: A laboratory study. Int Endod J. 2022;55:1091-1102.
[23] Dave AG, Mallya PL, Ballal NV, Shenoy R. Effect of ethylenediaminetetraacetic acid, maleic acid, and fumaric acid on post-endodontic treatment root fracture toughness – An in vitro study. JCD. 2023;26(4):453-57.
[24] Dogan Buzoglu H, Calt S, Gümüsderelioglu M. Evaluation of the surface free energy on root canal dentine walls treated with chelating agents and NaOCl. Int Endod J. 2007;40:18-24.
[25] Dotto L, Sarkis Onofre R, Bacchi A, Rocha Pereira GK. Effect of root canal irrigants on the mechanical properties of endodontically treated teeth: A scoping review. J Endod. 2020;46:596-604.
[26] Uzunoglu E, Yilmaz Z, Erdogan O, Gorduysus M. Final irrigation regimens affect fracture resistance values of root-filled teeth. J Endod. 2016;42(3):493-95.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 27, 2025
- Manual Googling: Oct 13, 2025
- iThenticate Software: Oct 18, 2025 (10%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 20, 2025**
Date of Peer Review: **Aug 08, 2025**
Date of Acceptance: **Oct 21, 2025**
Date of Publishing: **Jan 01, 2026**