#### **ORIGINAL RESEARCH**





# Prevalence of periapical radiolucency in endodontically treated teeth with untreated canals by CBCT

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## **Abstract**

Background: An untreated root canal in an endodontically treated tooth can lead to periapical lesions which can ultimately result in necrosis and inflammation of the pulp or destruction of periodontal tissues. This study was conducted to determine the prevalence of periapical radiolucency in endodontically treated teeth with untreated canals identified by CBCT.

Materials and Methods: In this analytical cross-sectional observational study, a total of 326 maxillary and mandibular premolars and molars with 775 root canals with previous root canal treatment obtained from CBCT images from the archives of the Radiology Center of the Faculty of Dentistry of Azad University of Isfahan (Khorasgan) were examined. The study recorded the number of teeth and roots, presence/absence of periapical lesions, and untreated canals. Data were analyzed using chi-square and Fisher's exact tests ( $\alpha$ =0.05).

Results: A total of 38 cases (4.9%) showed untreated canals, with the second mesiobuccal canal being the most common type (57.9%) and the maxillary first molar having the highest number of untreated canals (52.6%). In 125 canals (16.1%), apical periodontitis lesions were detected. There was a significant difference between the frequency of untreated canals in the endodontically treated maxillary premolars and molars, mandibular premolars, and molars (p<0.05). Similarly, there was a significant difference in the frequency of apical periodontitis between endodontically treated maxillary premolars and molars, and mandibular premolars and molars (p<0.001).

Conclusion: Apical periodontitis is more common in the second mesiobuccal canal of maxillary first molars that have not undergone successful root canal treatment.

Keywords: Cone-beam computed tomography; Root Canal Therapy; Periapical periodontitis

## Introduction

Adequate disinfection of the root canal system is one of the most crucial stages of root canal treatment (1). Several factors including persistent bacterial infection (2), inadequate root filling (3), untreated canals (4), inadequate coronal seal or inadequate crown reconstruction (5), and operative errors (6) are likely

to be associated with the persistence of apical periodontitis (7). Untreated canals can provide a favorable environment for the growth of microorganisms, resulting in apical periodontitis (8), which can affect the treatment outcome (9,10) Timely identification and diagnosis of periapical lesions is crucial for successful treatment and prognosis. General dentists play a key role in identifying and treating these lesions or referring patients to specialists if necessary.

Radiology is an essential aspect in root canal treatment, from diagnosis to treatment planning and evaluation of treatment success. Periapical radiography was commonly used in the past to observe apical periodontitis after root canal treatment. However, the limitations of this technique in searching

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for missed canals due to its 2D nature have been noted (13, 14). Despite being the most widely used method for evaluating root canal anatomy in clinical practice, periapical images cannot accurately depict the complex anatomy of the tooth (15).

Cone beam CT imaging provides an accurate 3D representation of teeth, jaw, and facial skeletal area and the relationship of anatomical structures (16). It has made visualization of pulp anatomy easier and, facilitated the accurate interpretation of root anatomy (17), including the number of canals and their possible curvatures, as well as the assessment of postoperative prognosis, particularly important in endodontic treatment (18-20) Advances in CBCT imaging have made it possible to assess the prevalence of untreated canals in endodontically treated teeth and their association with persistent periapical lesions Therefore, CBCT can be a powerful tool in root canal diagnosis as well as in treatment planning and follow-up.

Studies have shown that non-endodontically treated teeth have a 25.6 times higher possibility of periapical lesions than other teeth. The mesiobuccal root of the maxillary molar has the highest percentage (62.8) among the untreated roots and the second mesiobuccal canal of the maxillary molar has the highest percentage of the untreated canal (93%) among the dental canals, associated with periapical lesions in 75.2% of cases (21, 22).

In cross-sectional studies, improper treatments have been identified as a significant risk factor for apical periodontitis. Research has demonstrated that teeth with properly filled canals have a lower incidence of periapical lesions compared to those with improperly filled canals (23) an untreated root canal in an endodontically treated tooth can be due to the physician's limited knowledge of dental anatomy, complications of the root canal system, or operative errors like inadequate access cavity design). This study aimed to evaluate the prevalence of untreated root canals with periapical lesions in endodontically treated teeth.

## **Materials and Methods**

For this study, we conducted a retrospective cross-sectional observational analysis of 326 premolars and molars from the Faculty of Dentistry at Isfahan Azad University (Khorasgan) in 2022. We examined 775 root canals that had undergone endodontic treatment and were visible on CBCT images from the Archives of the Radiology Center. The primary objective of this study was to determine the prevalence of missed

canals and periapical radiolucency in these teeth.

To ensure accurate results, we only included teeth that had undergone endodontic treatment and had at least one visible root. We excluded images of third molars, teeth with incurable roots, impacted teeth, milk teeth, permanent teeth with immature apex, images with artifacts, the presence of a broken perforation file, root fracture and resorption, and teeth with improper coronal restoration and coronal seal.

The images were prepared using Galileos device (Sirona, Germany, Bensheim) under exposure conditions of 85 kV, 21 to 35 mAs, and FOV of 15 x 15 cm, and then were checked and measured in Sidexis 3D software.

The presence of untreated canals in endodontically treated teeth in all three dental axial, sagittal, and coronal planes were evaluated by a radiologist.

The following data was recorded for each tooth/root:

- 1. The number of the tooth and the number of its roots
- 2. Presence/absence of periapical lesion
- 3. Presence/absence of untreated canals

The collected data were analyzed using chi-square and Fisher's exact tests in SPSS ver. 26. A P-value=0.05 was considered as the significance level in this study.

### Results

The results revealed that most teeth examined (39.9%) had three canals. It was observed that 172 teeth were positioned on the left side and 154 teeth were positioned on the right side. The study further found that the maxillary first molar had the highest prevalence (16.9%) on the left side, while the mandibular first molar was the most commonly observed tooth on the right side (18.2%).

Among the 775 canals, 4401 were on the left side and 374 on the right side. The first maxillary molars (23.9%) and the first mandibular molars (24.3%) had the highest number of canals on the left and right sides, respectively. Untreated canals were observed in 38 canals (4.9%). The most common type of untreated canal was the second mesiobuccal canal (57.9%) and the maxillary first molars had the most untreated canal (52.6%).

Apical periodontitis lesions were observed in 125 canals (16.1%). The most common type of canal affected was the mesiobuccal canal (36.8%). Most of the affected teeth were maxillary first molars, which accounted for 35.2% of the total affected teeth.

The Chi-square test was used to compare the frequency of untreated canals in the studied teeth. The results showed a significant difference between maxillary premolars, mandibular first premolars, and molars in terms of the number of untreated canals (p=0.005). This means that the proportion of untreated canals in maxillary first molars was significantly higher than in maxillary premolars, mandibular first premolars, and molars. Additionally, there was a significant difference between second maxillary and mandibular premolars

and molars in terms of the number of untreated canals (p=0.005). Specifically, the proportion of untreated canals in maxillary second molars was significantly higher than in maxillary premolars, mandibular second premolars, and molars (Table 1).

Table 1. Comparison of untreated canal frequency in the maxillary premolar and molar teeth, root-treated mandibular premolar, and molar teeth

Tooth	untreated canal	maxillary premolar	maxillary molar	Mandibular premolar	Mandibular molar	P value
		No (%)	No (%)	No (%)	No (%)	1 value
First	without	89(96.7)	164 (89.1)	28(100.0)	174(96.7)	
	with	3(3.3)	20(10.9)	0(0.0)	6(3.3)	0.005
	total	92(100.0)	184(100.0)	28(100.0)	180(100.0)	
Second	without	85(98.8)	70(90.9)	38(100.0)	89(98.9)	
	with	1(1.2)	7(9.1)	00.0 ()	1(1.1)	0.005
	Total	86(100.0)	77(100.0)	38(100.0)	90(100.0)	

The study findings revealed a considerable difference in the prevalence of apical periodontitis between maxillary and mandibular first premolars and molars (p=0.001). The prevalence of apical periodontitis in the maxillary first molars and mandibular first molars were significantly more than maxillary premolars, mandibular first premolars (p<0.05). There was no

significant difference between maxillary first molars and mandibular first molars in terms of the prevalence of apical periodontitis (p<0.05). The mandibular first premolar had the lowest periapical lesion, and the maxillary first molar had the highest periapical lesion among the studied teeth (Table 2).

Table 2. Comparison of the frequency of apical periodontitis in root-treated maxillary premolar and molar, mandibular premolar, and molar teeth

Tooth	Apical periodontitis	maxillary premolar	maxillary molar	Mandibular premolar	Mandibular molar	P value	
		No (%)	No (%)	No (%)	No (%)	1 value	
First	No AP	86 (93.5)	140 (76.1)	27 (96.4)	143 (79.4)		
	With AP	6 (6.5)	44(23.9)	1(3.6)	37(20.6)	0.001	
	Total	92 (100.0)	184(100.0)	28 (100.0)	180(100.0)		
Second	No AP	80 (93.0)	67(87.0)	34 (89.5)	73(81.1)	0.121	
	With AP	6 (7.0)	10(13.0)	4 (10.5)	17(18.9)	0.121	

Furthermore, the research indicates a significant relationship between missed canal and apical periodontitis in the studied teeth The occurrence of apical periodontitis was found in 89.5% of the missed canals and 12.3% of the treated canals. It suggests a significant

difference between the presence of the missing canal and the presence of apical periodontitis (p<0.001). The prevalence of lesions was significantly higher in the missed canals. However, mandibular premolars did not exhibit any missed canals (Table 3).

**Table 3.** The relationship between lost canal and apical periodontitis in root-treated maxillary premolars and molars, mandibular premolars, and molars

	Apical periodontitis	No missing canal	Missing canal	P value	
	Apical periodolitius	No (%)	No (%)	r value	
	No AP	166 (95.4)	0(0.0)		
maxillary premolar	With AP	8(4.6)	4(100.0)	< 0.001	
	total	174(100.0)	4(100.0)		
	No AP	203(86.8)	4(14.8)	< 0.001	
maxillary molar	With AP	31(13.2)	23(85.2)		
	total	233(100.0)	27(100.0)		
	No AP	61(92.4)	0(0.0)		
Mandibular premolar	With AP	5(7.6)	0(0.0)		
	total	66(100.0)	-(-)		
	No AP	216(82.1)	0(0.0)		
Mandibular molar	With AP	47(17.9)	7(100.0)	< 0.001	
	total	263(100.0)	7(100.0)		

## Discussion

The present study found that most of the teeth that received endodontic treatment were the first molars in both the upper and lower jaws the frequency of periapical lesions in these teeth was reported as 16.1%. However, other studies conducted by Deyhimi et al. (24), Jamshidi (25), and Al-Omari et al. (26) reported varying rates of periapical lesions, specifically 5.44%, 18.3%, and 83.7%, respectively Notably, only the study by Jamshidi et al. (25) demonstrated results that were comparable to those of the present study. This disparity suggests that the frequency of periapical lesions in endodontically treated teeth is subject to variation within different populations, which may be attributed to differences in the diagnostic capabilities of CBCT imaging.

Based on the findings, the prevalence of apical periodontitis was highest in maxillary molars, mandibular molars, mandibular premolars, and maxillary premolars in that order. The maxillary molars showed the highest number of periapical lesions, specifically in the second mesiobuccal canal. Furthermore, it was observed that the most missed canals were in maxillary molars affected by apical periodontitis.

In other words, the failure to identify the mesiobuccal canal in maxillary molar teeth, which is highly prevalent (27), leads to insufficient root filling, and thereby doubles the risk of apical periodontitis. It is clear that with an increase in the number of canals, the risk of missed root canals also increases (28) Based on previous studies, the absence of or insufficient root filling can contribute to apical periodontitis and it is considered the main cause of the condition (11, 23, 29-31). The health of the peri-apical region is impacted by a multitude of factors, such as its condition prior to pulp treatment, the complexity of the tooth's canal system, and the root treatment process. This process encompasses shaping and cleaning procedures, canal disinfection, washing protocols, humidity control and the quality of the coronal restoration (32,33).

According to a study by Sunay et al. (34), 91% of teeth with low treatment quality had apical periodontitis. In another study, Ödesjö et al. (35) found that apical periodontitis was more commonly associated with teeth that had undergone endodontic treatment. A study by Kirkevang et al. (36) revealed that teeth with sufficient root filling length had a lower prevalence of peri-apical lesions compared to those with inadequate length. Therefore, it can be concluded that there is a

positive relationship between inappropriate filling length and the presence of periapical lesions.

The results of the present study showed that the maxilla was more affected than the mandible, which is consistent with previous research (37, 38). However, many studies considered mandibular molars as teeth that are strongly associated with the presence of periapical lesions (39, 40). Since the mandibular first molars are one of the first teeth that grow in the permanent tooth system, they are more exposed to caries and pulp diseases (40).

According to a study conducted by Baruwa et al (22), the highest occurrence of periapical lesions was found in the mesiobuccal root of the first molar in the upper jaw. In a study by Costa et al. (21), the risk of apical periodontitis was revealed 25.6 times higher in endodontically treated teeth. They also discovered that the highest frequency of untreated canals was related to the mesiobuccal roots of maxillary first molars, whilst the highest missed canals occurred in the second mesiobuccal canal. The study concluded that endodontically treated teeth with at least one missed canal had a high prevalence of apical periodontitis after treatment. do Carmo et al. (28) also concluded that when the number of root canals is greater, the risk of missing canals is higher, and the most missed canals are associated with the presence of periapical lesions endodontically treated maxillary According to a study conducted by Karabucak et al. (16), the prevalence of untreated canals was highest in maxillary molars (40.1%) and lowest in maxillary premolars (9.5%). Additionally, non-endodontically treated teeth had a 4.8 times higher frequency of periapical lesion. These findings are consistent with the results of the present study, indicating that missed canals significantly affect treatment prognosis by contributing to the presence of periapical lesions.

Lack of root filling or insufficient root filling (less than 2 mm) is the most significant risk factor for apical periodontitis. The risk of apical periodontitis increases significantly in endodontically treated teeth, especially in maxillary first molars, which have a second mesiobuccal canal that can be missed for various reasons, such as dentist's inaccuracy, image artifacts, and errors in examined images. Therefore, further studies of root canal images are required to identify the anatomy and structure of the root canals of the teeth, and to ensure the accuracy of root canal cleaning and filling in endodontically treated teeth.

### Conclusion

Periapical radiolucency is more common in the second mesiobuccal canal of maxillary first molars teeth that have not had successful root canal treatment.

**Conflict of Interests:** The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

### References

- Siqueira JF Jr, Magalhães KM, Rôças IN. Bacterial reduction in infected root canals treated with 2.5% NaOCl as an irrigant and calcium hydroxide/camphorated paramonochlorophenol paste as an intracanal dressing. J Endod. 2007;33(6):667-72.
- Nair PN, Sjögren U, Krey G, Kahnberg KE, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. J Endod. 1990;16(12):580-8.
- Alizade E, Ranjbarian P, Torkzadeh A, Shariati Najafabadi Saharsadat. Prevalence of Technical Errors in a Sample of Endodontically Treated Teeth: a CBC Analysis. J Res Dent Sci. 2023; 20(2):43-50
- 4. Nair PN. On the causes of persistent apical periodontitis: a review. Int Endod J 2006;39:249–81.
- Tronstad L, Asbjørnsen K, Døving L, et al. Influence of coronal restorations on the periapical health of endodontically treated teeth. Dent Traumatol 2000;16:218–21.
- 6. Siqueira Jr JF. Aetiology of root canal treatment failure: why well-treated teeth can fail. Int Endod J 2001;34(1):1–10.
- 7. Ng YL, Mann V, Gulabivala K. Outcome of secondary root canal treatment: a systematic review of the literature. Int Endod J 2008;41:1026–46.
- 8. Wolcott J, Ishley D, Kennedy W, et al. A 5 yr clinical investigation of second mesiobuccal canals in endodontically treated and retreated maxillary molars. J Endod 2005;31:262–4.
- 9. Cantatore G, Berutti E, Castellucci A. Missed anatomy: frequency and clinical impact. Endod Topics 2006;15:3–31.
- 10. Witherspoon DE, Small JC, Regan JD. Missed canal systems are the most likely basis for endodontic retreatment of molars. Tex Dent J 2013;130:127–39.
- 11. Petersson A, Axelsson S, Davidson T, Frisk F, Hakeberg M, Kvist T, et al. Radiological diagnosis of periapical bone tissue lesions in endodontics: a systematic review. International Endodontic Journal 2012; 45(9): 783-801.
- 12. Bjørndal L, Kirkevang LL, Whitworth JM. Textbook of Endodontology.3<sup>rd</sup> ed. Hoboken: Wiley, 2018.
- 13. Tsuneishi M, Yamamoto T, Yamanaka R, et al. Radiographic evaluation of periapical status and prevalence of endodontic treatment in an adult

- Japanese population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;100:631–5.
- 14. Siqueira JF Jr, Rôças IN, Alves FR, Campos LC. Periradicular status related to the quality of coronal restorations and root canal fillings in a Brazilian population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;100(3):369-74..
- 15. Fan B, Gao Y, Fan W, Gutmann JL. Identification of a C-shaped canal system in mandibular second molarspart II: the effect of bone image superimposition and intraradicular contrast medium on radiograph interpretation. J Endod. 2008;34(2):160-5.
- 16. Karabucak B, Bunes A, Chehoud C, Kohli MR, Setzer F. Prevalence of Apical Periodontitis in Endodontically Treated Premolars and Molars with Untreated Canal: A Cone-beam Computed Tomography Study. J Endod. 2016;42(4):538-41.
- 17. Salavati M, Torkzade A, Ranjbarian P, Hashemi Seyghalani Z. Prevalence and Morphology of the Middle Mesial Canal in the First and Second Mandibular Molars Using Cone-Beam Computed Tomography. JSSU 2023; 31 (5):6693-6704
- Versteeg KH, Sanderink GC, van Ginkel FC, van der Stelt PF. Estimating distances on direct digital images and conventional radiographs. J Am Dent Assoc. 1997;128(4):439-43.
- Mohammadi Z, Giardino L, Palazzi F, Shalavi S, Alikhani MY, Lo Giudice G, et al. Effect of sodium hypochlorite on the substantivity of chlorhexidine. International Journal of Clinical Dentistry 2013; 6(2):173–178.
- Lo Giudice G, Lizio A, Giudice RL, Centofanti A, Rizzo G, Runci M, et al. The Effect of Different Cleaning Protocols on Post Space: A SEM Study. Int J Dent. 2016;2016:1907124.
- 21. Costa FFNP, Pacheco-Yanes J, Siqueira JF Jr, Oliveira ACS, Gazzaneo I, Amorim CA, et al. Association between missed canals and apical periodontitis. Int Endod J. 2019;52(4):400-406.
- Baruwa AO, Martins JNR, Meirinhos J, Pereira B, Gouveia J, Quaresma SA, et al. The Influence of Missed Canals on the Prevalence of Periapical Lesions in Endodontically Treated Teeth: A Cross-sectional Study. J Endod. 2020;46(1):34-39.e1.
- Georgopoulou MK, Spanaki-Voreadi AP, Pantazis N, Kontakiotis EG, Morfis AS. Periapical status and quality of root canal fillings and coronal restorations in a Greek population. Quintessence Int. 2008;39(2):e85-92.
- Deihimy P, Khalesi S, Nazemi Behbahani L, Arefian MR. Periapical diseases and spread of odontogenic infections. J Isfahan Dent Sch 2014; 10(3): 276-99
- Jamshidi S, Shojaei S, Roshanaei G, Modabbernia S, Bakhtiary E. Jaw Intraosseous Lesions Biopsied Extracted From 1998 to 2010 in an Iranian Population. Iran Red Crescent Med J. 2015;17(6):e20374.

- Al-Omari MA, Hazaa A, Haddad F. Frequency, and distribution of root filled teeth and apical periodontitis in a Jordanian subpopulation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 111(1): e59-e65.
- 27. Esmaeilian A, Torkzadeh A, Mortaheb A, Zakariaee Juybari A. The Examination of Root Morphology of the Maxillary First and Second Molars Using Cone Beam Computed Tomography. J Isfahan Dent Sch 2021; 17(3): 329-336.
- do Carmo WD, Verner FS, Aguiar LM, Visconti MA, Ferreira MD, Lacerda MFLS, et al. Missed canals in endodontically treated maxillary molars of a Brazilian subpopulation: prevalence and association with periapical lesion using cone-beam computed tomography. Clin Oral Investig. 2021;25(4):2317-2323.
- 29. Silva BSF, Bueno MR, Yamamoto-Silva FP, Gomez RS, Peters OA, Estrela C. Differential diagnosis and clinical management of periapical radiopaque/hyperdense jaw lesions. Braz Oral Res. 2017;31:e52.
- 30. Razavi SM, Kiani S, Khalesi S. Periapical lesions: a review of clinical, radiographic, and histopathologic features. Avicenna Journal of Dental Research 2015; 7(1): 1-7.
- 31. Ali AH, Mahdee AF, Fadhil NH, Shihab DM. Prevalence of periapical lesions in non-endodontically and endodontically treated teeth in an urban Iraqi adult subpopulation: A retrospective CBCT analysis. J Clin Exp Dent. 2022;14(11):e953-e958
- 32. Ng YL, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. Int Endod J. 2011;44(7):583-609.

- 33. Sjogren U, Hagglund B, Sundqvist G, Wing K. Factors affecting the long-term results of endodontic treatment. J Endod. 1990;16(10):498-504.
- 34. Sunay H, Tanalp J, Dikbas I, Bayirli G. Cross-sectional evaluation of the periapical status and quality of root canal treatment in a selected population of urban Turkish adults. Int Endod J. 2007;40(2):139-45.
- 35. Odesjö B, Helldén L, Salonen L, Langeland K. Prevalence of previous endodontic treatment, technical standard and occurrence of periapical lesions in a randomly selected adult, general population. Endod Dent Traumatol. 1990;6(6):265-72.
- 36. Kirkevang LL, Ørstavik D, Hörsted-Bindslev P, Wenzel A. Periapical status and quality of root fillings and coronal restorations in a Danish population. Int Endod J. 2000;33(6):509-15.
- 37. Lupi-Pegurier L, Bertrand MF, Muller-Bolla M, Rocca JP, Bolla M. Periapical status, prevalence and quality of endodontic treatment in an adult French population. Int Endod J. 2002;35(8):690-7.
- 38. Spatafore CM, Griffin JA Jr, Keyes GG, Wearden S, Skidmore AE. Periapical biopsy report: an analysis of over a 10-year period. J Endod. 1990;16(5):239-41.
- 39. Buckley M, Spångberg LS. The prevalence and technical quality of endodontic treatment in an American subpopulation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995;79(1):92-100.
- 40. Yildirim D, Aydin U, Gormez O, Yilmaz HH, Bozdemir E, Aglarci OS, et al. Endodontically related lesions on panoramic radiographs in a Turkish subpopulation. Journal of Oral and Maxillofacial Radiology 2013; 1(1): 8-12