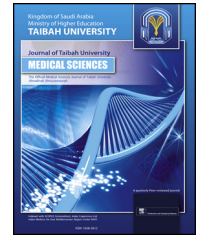




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Original Article

Relationship between apical periodontitis and missed canals in mesio-buccal roots of maxillary molars: CBCT study

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المخلص

أهداف البحث: تتضمن أهداف الدراسة: (1) تقييم مدى انتشار القنوات المفقودة في الجذور البوغيّة الوسطى للأسنان الطواحين العلوية المعالجة عن طريق العلاج الجذري والعلاقة بينها وبين التهاب اللثة المحيط بالجذر، (2) دراسة العلاقة بين وجود قناة البوغيّة الوسطى الثانية المفقودة المندمجة أو المنفصلة ومعدل انتشار التهاب اللثة المحيط بالجذر، (3) دراسة العلاقة بين الجودة التقنية للعلاج الجذري في قناة البوغيّة الوسطى الأولى ومعدل انتشار التهاب اللثة المحيط بالجذر.

طرق البحث: تم الحصول على ودراسة ٨٠٠ صورة من التصوير بالأشعة المقطعية المخروطية من سجلات ٨٠٠ مريض على مدى ستة أشهر. تضمنت المعلومات الملاحظة لكل سن: (أ) رقم السن، (ب) وجود قناة مفقودة في الجذر البوغي الوسطى، (ج) تشكيل القناة البوغيّة الوسطى الثانية المفقودة (مندمجة أو منفصلة)، (د) قبولية العلاج الجذري من الناحية التقنية للقنوات البوغيّة الوسطى الأولى المعالجة، (هـ) نتيجة المؤشر الشعاعي الجذري على التصوير بالأشعة المقطعية المخروطية.

النتائج: تم اختيار ٢٠٣ طاحنة علوية من ١٤٨ صورة من التصوير بالأشعة المقطعية المخروطية. كانت معدلات انتشار القناة البوغيّة الوسطى الثانية ٨٨,٢٪ في الأسنان الطواحين العلوية الأولى و٦٢,٧٪ في الأسنان الطواحين العلوية الثانية. تم العثور على القناة البوغيّة الوسطى الثانية في ١٦٤ من الأسنان الطواحين العلوية المعالجة من خلال العلاج الجذري. خلال العلاج، تم تفويتها في

١٥٠ سن (٩١,٥٪) وتم علاجها في ١٤ سن (٨,٥٪). أظهرت الدراسة أن 100٪ من الأسنان التي تم تفويتها في العلاج الجذري لها التهاب لثة محيط بالجذر، بينما ٣٥,٧٪ من الأسنان التي تم علاجها تحتوي على التهاب لثة محيط بالجذر. ظهرت الفروق في الانتشار كفروق ذات دلالة إحصائية. كانت القناة البوغيّة الوسطى الثانية المفقودة المندمجة أكثر انتشاراً (٥٥,٢٪) من القناة البوغيّة الوسطى الثانية المفقودة المنفصلة (٣٣,٥٪). كان معدل انتشار التهاب اللثة المحيط بالجذر أعلى في الأسنان التي كانت تحتوي على قناة بوغيّة وسطى ثانية مندمجة (١٠٠٪) مقارنة بالأسنان التي كانت تحتوي على قناة بوغيّة وسطى ثانية منفصلة (٤٠٪). كانت الفروق في الانتشار تحمل دلالة إحصائية.

الاستنتاجات: يبدو أن هناك علاقة قوية بين تفويت القناة البوغيّة الوسطى الثانية في أسنان الطواحين العلوية المعالجة عن طريق العلاج الجذري ووجود التهاب اللثة المحيط بالجذر. يتطلب الكشف والعلاج الصحيح للقناة البوغيّة الوسطى الثانية مهارة ومعرفة كبيرتين من قبل أطباء الأسنان لتجنب التهاب اللثة المحيط بالجذر.

الكلمات المفتاحية: التصوير بالأشعة المقطعية المخروطية؛ علاج الجذر؛ سن الطاحونة العلوية؛ الجذر البوغي الوسطى؛ الصحة القوية

Abstract

Objectives: The objectives of this study were to: (1) assess the frequency of missed canals in the mesiobuccal root (MB) of endodontically treated maxillary molars and its association with apical periodontitis (AP); (2) examine the correlation between the presence of a confluent or separate missed MB2 canal and the prevalence of AP; and (3) examine the correlation between the technical quality of endodontic treatment in the MB1 canal and the prevalence of AP.

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Methods: We obtained and examined 800 cone-beam computed tomography (CBCT) scans from 800 patient records over 6 months. The parameters noted for each tooth included the tooth number; presence of missed canals in the MB root; configuration of missed MB2 canals (confluent or separate); technical acceptability of root canal treatment (RCT) of treated MB1 canals; and the CBCT periapical index score. Data were analyzed in SPSS version 24.

Results: A total of 203 maxillary molars from 148 CBCT scans were included. The MB2 canal prevalence was 88.2% in maxillary first molars and 62.7% in maxillary second molars. MB2 was found in 164 endodontically treated maxillary molars. During treatment, MB2 was missed in 150 (91.5%) and treated in 14 (8.5%) teeth. A total of 103 teeth (50.73%) had AP, which was observed in 67.3% of teeth with a missed MB2 canal but only 14.3% of teeth with a treated MB2 canal. The prevalence of AP was 43.7% in teeth with confluent MB2 canals and 80.9% in teeth with separate MB2 canals.

Conclusion: The MB2 canal frequency was significantly higher in the examined maxillary first molars than the maxillary second molars. The MB2 canal was missed in most teeth that underwent endodontic treatment. The AP prevalence was relatively higher in endodontically treated maxillary molars with missed MB2 canals.

Keywords: Cone beam computed tomography; Endodontics; Maxillary molar tooth; Mesio-buccal root; Oral health

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Introduction

Adequate root canal system (RCS) disinfection is integral to the long-term success of endodontic treatment (ET).¹ ET failure has been attributed to numerous factors, including inadequate RCS debridement and/or filling,² missed canals,³ unsatisfactory coronal restoration⁴ and iatrogenic procedural errors.⁵ Some of the most common causes of missed root canals during ET are limited knowledge of root canal morphology, lack of clinician experience, inappropriate access to the RCS, inadequate pre-operative imaging and assessment of RCS anatomy, and teeth with unusual or complex morphological features. The untreated root canal may serve as a reservoir for pathogenic bacteria. This reservoir effect is a major etiological factor in persistent apical periodontitis (AP) and resultant ET failure.⁶

Previous research on post-ET apical periodontitis has been conducted with conventional intra-oral periapical radiography (IOPR).^{7–10} Conventional IOPR has many limitations, such as a lack of the third dimension and superimposition of adjacent anatomical structures. In

addition, differing attenuation between the cortical and cancellous bones may conceal minor changes in the periapical area in IOPR.¹¹ Cone-beam computed radiography (CBCT) is a major diagnostic advancement in dentistry that provides a three-dimensional image of the oral and maxillofacial region.^{12–15} Many studies have shown that CBCT is accurate and highly sensitive for the assessment of RCS and detection of AP.^{16–19}

The mesiobuccal (MB) root of maxillary molars (MM) is the most difficult root to treat during ET, because of the curvature and complexity of its root canals. The frequency of additional canals in the MB roots of maxillary first molars (MFM) has been reported to range between 18% and 97.6%, with a pooled prevalence of 69.6% depending on the method used, whereas additional root canals in the MB roots of maxillary second molars (MSM) are found in approximately 5%–70% of cases, with a pooled prevalence of 39%.^{7–9,20–22} In the Saudi population, the prevalence of additional canals in the MB roots of MM has been reported to range from 32.3% to 97% for MFM, and 19.7%–93% for MSM.^{10,23–27} Therefore, clinicians often inadvertently leave a canal untreated in that root. Baruwat et al.²⁸ have found that the MB roots of MFM and MSM have the highest frequency of missed root canals in endodontically treated teeth, on the basis of CBCT assessment (62.8% and 49%). Consequently, the same root has the highest prevalence of AP (75.2% and 68%, respectively). Mashyakhly et al.²⁹ have examined 208 CBCT samples in the Jizan region (KSA) and have found the highest prevalence of missed canals in MFM (40.7%, compared with 16.7% in MSM). They have also found a high prevalence of AP among those teeth. However, their study involved a relatively small sample size, and only 44 endodontically treated MM were examined.

Although the success rate of non-surgical ET is relatively high, and the prognosis is usually predictable, failure is possible, as with any other medical procedure, and post-treatment disease may result.³⁰ ET is considered a success when it alleviates pain, causes resolution of periapical infection and prevents re-infection. When these goals are not achieved after endodontic therapy, the procedure is considered a treatment failure. One of the most frequent reasons for ET failure is a missed canal.^{31,32} Missed canals result in incomplete removal of infection-causing bacteria, which survive in the missed canal, spread to the periapex and cause apical periodontitis.^{33,34}

To our knowledge, no study in the literature has focused specifically on the MB roots of MM to assess the frequency of missed root canals and their association with the prevalence of AP, while also considering the root canal configuration and obturation quality of the treated canal. Moreover, no study conducted in the Qassim region has assessed the association between missed canals and the prevalence of AP among endodontically treated teeth. Therefore, this study was aimed at (1) assessing the frequency of missed canals in the MB roots of endodontically treated MFM and MSM, and its association with the prevalence of AP; (2) examining the correlation between the presence of a confluent or separate MB2 canal and the prevalence of AP; and (3) examining the correlation

between the technical quality of root canal treatment in MB1 canal and the prevalence of AP.

Materials and Methods

The study was conducted at the Department of Endodontics, College of Dentistry, Qassim University. Before the beginning of the study, a pilot study was conducted on 20 samples. On the basis of the prevalence in the pilot study, the sample size was calculated in G*Power software. A minimum sample size of 113 teeth was calculated to be required, with an effect size of 0.34, 95% power and an error probability of 0.05. Overall, 800 CBCT scans of Saudi Patients were screened in the study over a 6-month period (September 2022 to March 2023). The scans were obtained by screening for nationality in the patients' files from the archives of the Qassim University Dental Clinic oral radiology department. A total sample of 203 MM teeth in 148 CBCT scans that met the inclusion criteria were included in this study. Patients referred for various diagnostic purposes, such as implant placement, impaction, orthodontic therapy or ET, were included. The inclusion criteria were endodontically treated permanent MFM and MSM with acceptable coronal restorations, and images of good quality. Teeth with defective coronal restorations, open apices, fused roots, remaining roots, C-shaped canals or unclear images were excluded from the study. Endodontically treated MM with untreated MB roots were also excluded. The criteria for good-quality ET were extension of the root filling to within 2 mm of the radiographic apex, the absence of voids, and the absence of procedural errors such as perforations and ledges.

Data collection

All scans were obtained from the same CBCT machine Galileos (Dentsply Sirona, Biensham, Germany) with scan parameters of 98 kV, 3–6 mA, scan time/exposure time of 14 s/2–5 s, a field of view of 15 × 8.5 cm collimated and a voxel size of 0.16 mm. The images were analyzed in the proprietary software Sidexis 4. Image evaluation was performed by two independent observers (one endodontist and one oral radiologist, each with more than 5 years of experience). The axial, sagittal and coronal planes were aligned to vertical and horizontal lines parallel to the long axis of the MB root, and evaluation was performed in the axial and sagittal sections. Each MB root was traced axially from the canal orifice to the apex, as seen on the scan. For recording an MB2 canal, tracing was performed in successive sections to at least half the length of the root. Periapical status was assessed in the sagittal section. Each observer performed a step-by-step predefined assessment to improve the reliability, after joint training on ten selected random scans. Examinations were performed independently by observers blinded to the patient data, then repeated after a 3-week interval. The readings of the two

observers were compared. In the event of disagreement, the case was discussed until a consensus was reached. The following parameters were noted for each tooth: tooth number; presence of a missed root canal in the MB root (yes/no); merging of the missed root canal with the treated canal (yes/no); technically acceptable ET in the treated canal (yes/no); and CBCT periapical index score. The periapical status of the endodontically treated MB root was scored with the CBCT periapical index score as described by Estrela et al.³⁵ The absence of a lesion was indicated by a score of 0 (intact periapical bone structures), whereas the presence of disease was indicated by scores ranging from 1 to 5 (diameter of periapical radiolucency above 0.5 mm).

Statistical analysis

The data were analyzed in SPSS version 24 software (IBM Corp, Armonk, NY). We conducted intra- and inter-examiner reliability evaluations. Cohen's kappa test was used to ascertain intra-examiner consistency. The first 50 endodontically treated MM were examined twice (observations A and B) in a 2-week interval, and the findings were evaluated. On the basis of the inter-examiner correlation coefficients on these 50 teeth, the inter-examiner reliability for observations A and B was determined. If the measured kappa coefficient of agreement and intra-examiner correlation coefficient values exceeded 0.60, the group and individual observers were deemed reliable. The chi-square test was used to compare different variables regarding missed or treated MB2 in relation to the prevalence of AP. The Pearson correlation test was used to analyze the correlation of confluent or separate MB2 with AP. The significance threshold was set at a p-value <0.05.

Results

The Kappa test revealed an intra-examiner reliability of 0.85 and inter-examiner reliability of 0.80; therefore, both were deemed reliable. A total of 203 MM in 148 patients met the study inclusion criteria (#16, 76 teeth; #17, 30 teeth; #26, 68 teeth; and #27, 29 teeth). The overall incidence of the MB2 canal was 88.2% in MFM and 62.7% in MSM (Figures 1 and 2). Further analysis was conducted on 164 endodontically treated MM with MB2 present. The prevalence of missed MB2 among these teeth is illustrated in Table 1. When MB2 was present, MB2 was missed in 150 (91.5%) and treated in 14 (8.5%) of 164 teeth. Table 2 shows the prevalence of AP in all MFM and MSM (203 teeth). A total of 103 teeth (50.73%) had AP.

Table 3 compares the correlation of missed or treated MB2 canals with the prevalence of AP. AP was observed in 67.3% of teeth with a missed MB2 canal but only 14.3% of teeth with a treated MB2. Analysis with the chi-square test indicated that the prevalence of AP was significantly higher in teeth with a missed MB2 canal compared to teeth with a treated MB2 canal (p-value < 0.0001).

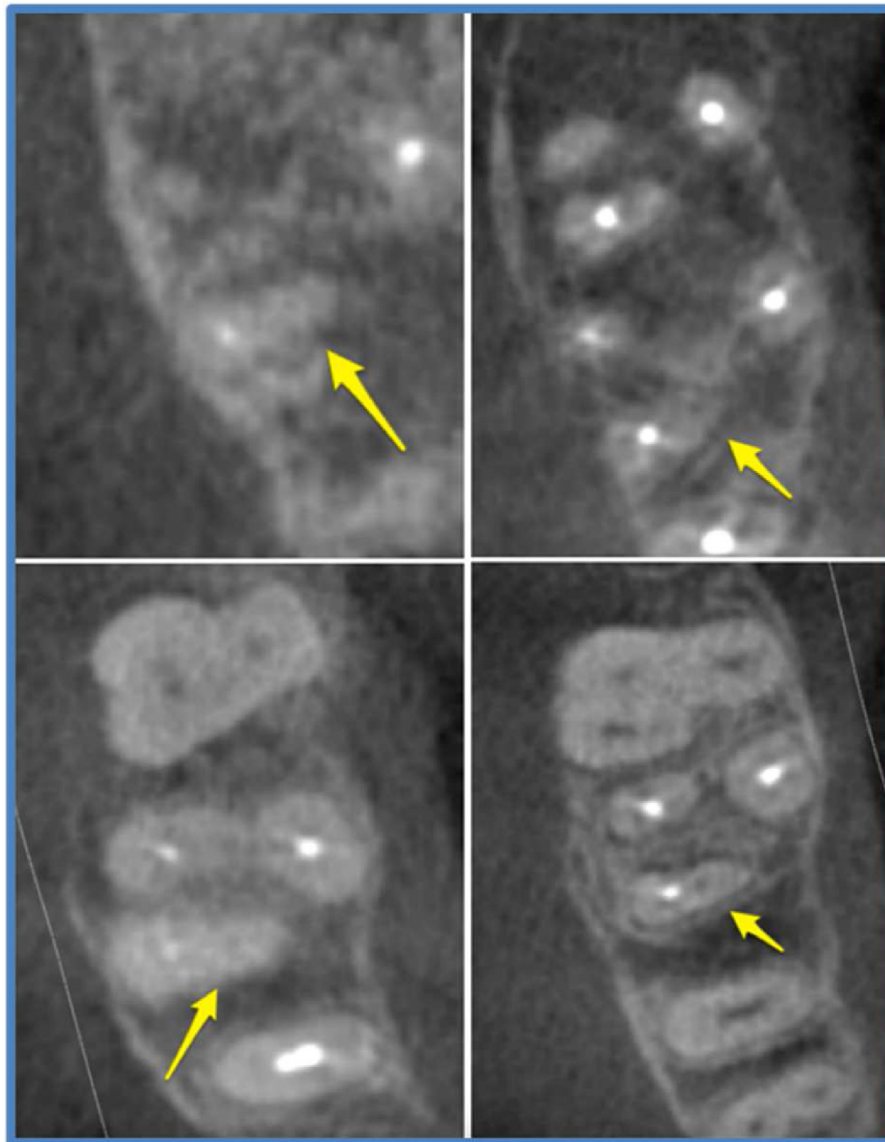


Figure 1: Examples of missed MB2 canals in maxillary first molars (axial plane).

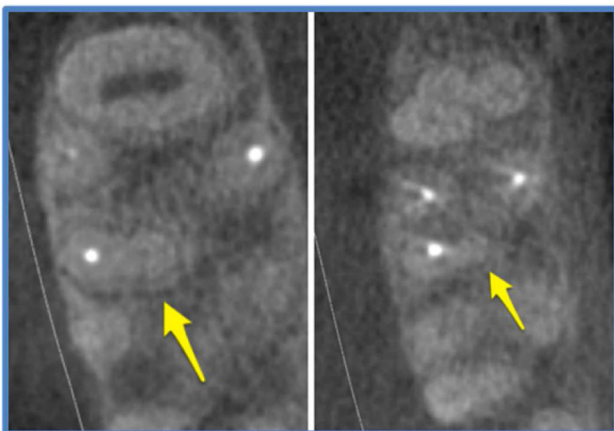


Figure 2: Examples of missed MB2 canals in the second maxillary molar (axial plane).

The influence of a confluent or separate MB2 canal is shown in [Table 4](#). The prevalence of AP was 43.7% when a confluent MB2 canal was present ([Figure 3](#)) and 80.9% when a separate MB2 canal was present ([Figure 4](#)). Statistical analysis indicated a greater likelihood of development of apical periodontitis in teeth with a separate MB2 canal than in teeth with a confluent MB2 canal ($r=0.645$; $p = 0.013$).

The influence of the quality of ET of the MB1 canal in relation to the prevalence of AP is shown in [Table 5](#). Among teeth with MB2 present (164 teeth), 52.4% had a technically unacceptable root canal filling in the MB1 canal. The prevalence of AP was 33.3% when the MB1 had a technically acceptable root canal filling and 89.5% when a technically unacceptable root canal filling was placed in the MB1 canal. Statistical analysis showed that the prevalence of AP was significantly higher when the MB1 canals had technically unacceptable ET compared to those with technically acceptable ET ($p\text{-value} < 0.0001$).

Table 1: Prevalence of missed MB2 canal in maxillary molars, total and by tooth type (16, 17, 26, 27).

Tooth number	Total MB2 present N (%)	Total number of missed MB2 among all MB2 N (%)	Total number of treated MB2 N (%)	Prevalence of missed MB2 (%)
All molars	164 (100%)	150 (91.5%)	14 (8.5%)	91.5%
16 (Right maxillary first molar)	70	64 (91.4%)	6 (8.6%)	91.4%
17 (Right maxillary second molar)	19	17 (89.5%)	2 (10.5%)	89.5%
26 (Left maxillary first molar)	57	53 (92.9%)	4 (7.01%)	93%
27 (Left maxillary second molar)	18	16 (88.8%)	2 (11.2%)	88.8%

Table 2: Prevalence of apical periodontitis in endodontically treated maxillary molars.

Total number of root canal treated maxillary molars N (%)	Total number of maxillary molars with apical periodontitis N (%)	Prevalence (%)
203 (100%)	103 (50.73)	50.73%

Table 3: Comparison of missed and treated MB2 with the prevalence of apical periodontitis.

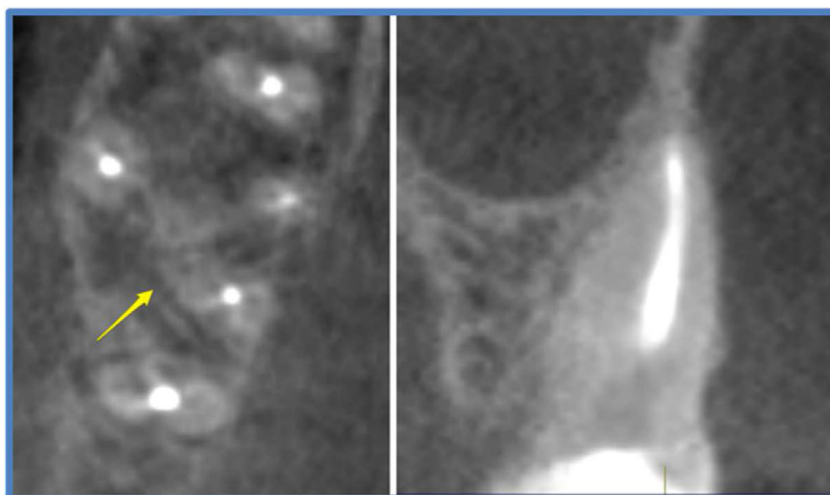
	Missed MB2 N (%)	Treated MB2 N (%)	Total N (%)
Apical periodontitis present	101 (67.3%)	02 (14.3%)	103 (62.8%)
Apical periodontitis absent	49 (32.7%)	12 (85.7%)	61 (37.2%)
Total	150 (100%)	14 (100%)	164 (100%)

Chi-square value 15.42; df 1; p-value <0.0001, highly significant.

Table 4: Prevalence of apical periodontitis in maxillary molars when the missed MB2 canal was confluent with or separate from the MB1 canal.

	Confluent MB2 with treated canal N (%)	Separate MB2 N (%)	Total N (%)
Apical periodontitis present	35 (43.7%)	68 (80.9%)	103 (62.8%)
Apical periodontitis absent	45 (56.3%)	16 (19.0%)	61 (37.2%)
Total	80 (100%)	84 (100%)	164 (100%)

Chi-square value 24.27; df 1; p-value <0.0001, highly significant.

**Figure 3:** A maxillary left first molar with a missed confluent MB2 canal but adequate RCT in the MB1 canal (apical periodontitis absent).

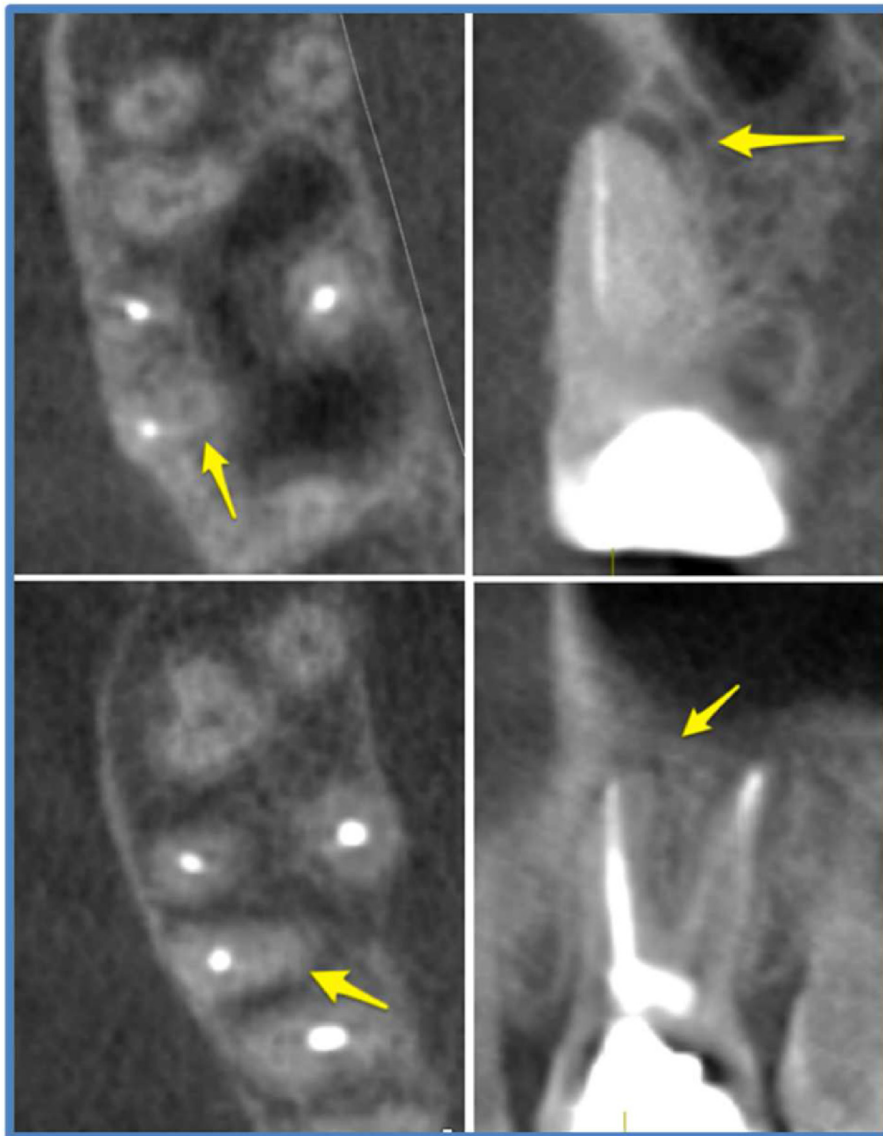


Figure 4: Maxillary right first molars with separate MB2 canals and adequate RCT in the MB1 canal (apical periodontitis present).

Table 5: Prevalence of apical periodontitis in maxillary molars with a missed MB2 canal when the quality of RCT in MB1 canal was adequate or inadequate.

	Technically adequate RCT in the MB1 canal N (%)	Technically inadequate RCT in the MB1 canal N (%)	Total N (%)
Apical periodontitis present	26 (33.3%)	77 (89.5%)	103 (62.8%)
Apical periodontitis absent	52 (66.6%)	09 (10.5%)	61 (37.2%)
Total	78 (100%)	86 (100%)	164 (100%)

Chi-square value 55.3; df 1; p-value <0.0001, highly significant.

Discussion

ET commences with the deroofting of the pulp chamber and removal of the coronal pulp, which is followed by the visualization of the pulpal floor and identification of the canal orifices. The practitioner must ensure that all canals are found during this procedure.³⁶ Therefore, for the success of ET,

clinicians must be fully aware of the anatomical norms and expected variations in the RCS of the tooth to be treated.^{12,30,37} The current study focused on the MB roots of MM and the number and frequency of MB canals that might be present but missed during ET. MM are morphologically variant and challenging to treat, and are often indicated for ET. Tooth morphology differs among populations of

different ethnicity, and the incidence of the MB2 canal in MM has been reported to vary among populations. In our study, the overall frequency of the MB2 canal was 88.2% in MFM and 62.7% in MSM. These findings are consistent with the results of similar studies conducted on Jordanian and Indian populations, which have found an MB2 canal in 87% and 86.36% of MFM, respectively.^{38,39} In contrast, a study assessing the RCS morphology of MFM in the Pakistani population has found presence of the MB2 canal in the MB root in 50% of teeth. This difference in findings might have been because that study was conducted in vitro and did not involve the use of CBCT imaging.⁴⁰ Similarly, in another study conducted on an Indian pediatric population, 20% of permanent MFM had MB2 canals.³⁶ Studies conducted on the RCS morphology of MSM in different populations have yielded varying results. One study conducted in India has reported a frequency of the MB2 canal in MSM of 29.4%, whereas a study conducted in Pakistan has reported a value of 21.52%.^{39,41} In a study by Mashyakhly, three roots were present in most MFM (98.9%); 48.7% of these MFM had three canals, whereas 46.4% had four canals.⁴² Another study conducted on the Saudi population has found an incidence of the MB2 canal in MFM and MSM of 46.7% and 17.7%, respectively.⁴³ Although those studies also used CBCT as an imaging modality, the difference in results is attributable to differences in sample size and study design. All these findings across studies worldwide indicate that RCS morphology is extremely complex and variable, thus potentially explaining why the MB2 canal in the MB roots of MM is the most frequently missed canal during ET.⁴⁴

In our study, of 164 endodontically treated teeth with MB2 present, the MB2 was missed in 150 (91.5%) and treated in only 14 (8.5%) teeth. Of the 150 teeth with missed MB2 canals, 101 (67.3%) teeth had associated apical periodontitis, whereas only 2 (14.3%) of 14 teeth with treated MB2 showed apical periodontitis. A similar study conducted in a Chilean subpopulation has reported 45.78% missed MB2 canals in MM and observed apical periodontitis in 70% of teeth with missed MB2 canals.⁴⁴ Similarly, a study in India has identified a missed MB2 canal in 77.19% of MFM and 90% of MSM, and apical periodontitis present in 72.7% of MFM and 88.8% of MSM.³⁹ Samantha et al. have reported 83.5% missed MB2 canals in MM, and apical periodontitis associated with 54.4% of these teeth.⁴⁵ The discrepancies in findings among studies may be explained by differences in the expertise of the observing clinicians, the methods and aids used in canal location and negotiation, and the research methods used. Another study has reported that the MB2 canal in the MB root of MFM is the most commonly missed canal during ET and has also demonstrated that 90% of teeth with missed canals are associated with apical periodontitis. However, that study was not exclusive to MM, and all posterior endodontically treated teeth were included in the study sample.⁴⁶ Despite the differences in numbers and percentages, all prior studies have indicated a notably high prevalence of apical periodontitis in MM with missed MB2 canals.

Although thorough disinfection of the canals is paramount to success, a key factor in determining the prognosis

of a root canal-treated tooth is the presence of an impermeable apical and coronal seal after obturation. An adequate coronal seal prevents any bacteria from surviving in the canals by blocking access to nutrients, and a good periapical seal stops bacteria from invading the peri-apical tissues.^{34,47} In the present study, the canal configuration and quality of obturation of the MB1 canal were also found to affect the treatment outcome. A total of 80.9% of Vertucci type IV canals showed apical periodontitis. In comparison, canals with type I and II configurations were associated with apical periodontitis in only 43.7% of cases. This finding might have been because, in some root canal configuration types, such as type II and III, two or more canals combine and exit the tooth apex via a single foramen, similarly to root canal configuration type I, in which only one apical foramen is present. In these situations, even if the other canal is inadvertently missed, an adequate apical and coronal seal of the treated canal may still prevent microorganisms from entering the apex and spreading infection. Other root canal configuration types, such as IV and VIII, possess several apical foramina, and each foramen must be correctly sealed to achieve endodontic effectiveness.⁴⁸ Periapical lesions can appear because of leakage from any of the apical foramina,³⁴ thus potentially explaining our findings regarding the quality of obturation of the treated MB canal. When a good quality seal through a technically acceptable root canal filling was present, apical periodontitis was observed in only 33.3% of cases of missed MB2 canals. However, when obturation of the MB1 was technically unacceptable, and MB2 was missed, apical periodontitis was observed in 89.5% of cases. These results highlight the importance of a good apical-coronal root canal seal.

The retrospective observational methods used in this study had the benefit of being less costly than longitudinal research. Observational studies also pose a diminished risk of bias. However, the shortcomings of this study include the CBCT voxel size used (0.16 mm). Smaller available voxel sizes are better suited for evaluating teeth during ET. In addition, a normal periodontal space may appear wider on CBCT, thus resulting in a misdiagnosis of apical periodontitis.⁴⁹ Comparison of preoperative and postoperative CBCT pictures in cross-sectional observational studies is impossible, because data are taken at one point in time. Consequently, whether a periapical lesion has healed, shrunk, or grown in size cannot be determined. Therefore, the appearance of periapical radiolucency or enlargement of the periodontal space alone cannot verify an endodontic failure. Another limitation of this study is that the sample was from only one center and one community; therefore, the results cannot be generalized to other populations. More research is necessary to better understand the relationships among missed root canals, canal configuration, root filling quality, and apical periodontitis. Future research should include a longitudinal study design with data collected from various centers and diverse ethnic populations. The results of this study should aid in treatment planning and clinical decision-making, as well as diagnosis of endodontic retreatment cases of permanent MM.

Conclusion

The MB2 canal prevalence was higher in MFM than MSM. The MB2 canal was left untreated in most teeth that underwent ET. Moreover, the prevalence of AP was relatively higher in endodontically treated MB roots with missed MB2 canals. Thus, the need to ensure that all root canals are found and adequately treated cannot be overemphasized. Thorough understanding of the internal architecture of the tooth enables teeth requiring ET to be successfully treated without accidentally missing any canals.

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Conflict of interest

The authors declare no conflicts of interest.

Ethical approval

The research protocol used in this retrospective study was approved by the ethical committee of Qassim University (reference no: 21-08-06).

Authors contributions

BA: conception and design of the study, data collection, analysis, preparation of tables and writing. MQJ and KIM: write-up and final editing/review of the manuscript. SDD and SSS: writing materials and methods, data collection and write-up. NMA: results analysis and final editing/review of the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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