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Evaluation of failed endodontically treated molar teeth using micro-computed tomography

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Abstract

Background The present study aimed to assess the internal root canal morphology and the quality of obturation in endodontically treated and failed first molars using micro-computed tomography (micro-CT).

Methods Forty-three mesial roots of extracted first molars with failed endodontic treatment were examined using micro-CT to evaluate root canal filling quality and root canal and isthmus configurations. The presence of untreated missed canals and the length of the root canal fillings were assessed. The percentage of voids within the canal filling in both apical and coronal regions was compared using an independent samples t-test. Statistical significance was set at $p < 0.05$.

Results An untreated second mesiobuccal canal was identified in 70.6% ($n = 12$) of the examined maxillary first molars, while a middle mesial canal was detected in 50.0% ($n = 13$) of the mandibular first molars. A significantly higher mean percentage of voids within the canal filling was observed in the apical region (15.3%) compared to the coronal region (7.3%) ($p < 0.05$). Short root canal fillings were observed in 57.7% of mandibular teeth and in 35.3% of maxillary teeth.

Conclusions The failure of endodontically treated teeth was associated with poor root canal filling quality and the presence of untreated extra canals, as revealed by micro-CT.

Keywords Micro-computed tomography, Endodontic failure, Root canal filling, Missed canals, First molars

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Introduction

Endodontic treatment is a cornerstone of dental care, aiming to preserve natural teeth affected by pulpal and periapical pathologies. Despite advances in endodontic materials and techniques, treatment outcomes are not always predictable, and failures still occur [1, 2]. A large-scale epidemiological study reported that within five years following root canal treatment, 12.1% of teeth experienced complications, of which 36.0% underwent non-surgical retreatment, 2.5% received apical surgery, and 61.5% were extracted [3].

In the general adult population, the prevalence of apical periodontitis has indicated a time-dependent increase in both endodontically treated (35.9% to 41.3%) and untreated teeth (2.1% to 3.5%) [4]. A systematic review and meta-analysis reported the global prevalence of apical periodontitis as 39.0% in endodontically treated and 3.0% in untreated teeth [5]. Similarly, cross-sectional studies have observed apical periodontitis in approximately 40.0% of endodontically treated teeth [6], with a significant association between periapical radiolucencies and inadequate endodontic treatment [7, 8]. These findings suggest that overall treatment outcomes remain sub-optimal [2], potentially due to insufficient adherence to clinical guidelines such as those of the European Society of Endodontology [9].

Endodontic failures can result from various factors, including inadequate canal debridement, procedural errors, poor coronal sealing, and persistent intraradicular infections [6]. Such infections are often linked to complex root canal anatomies, such as untreated extra canals, accessory canals, and isthmuses, that may not be fully instrumented or disinfected during treatment [10, 11]. Mechanical preparation may leave untouched areas within these complex anatomies, contributing to persistent apical inflammation and treatment failure [12]. Thus, a thorough understanding of root canal morphology is essential for improving treatment outcomes.

Micro-computed tomography (micro-CT) has emerged as the gold standard for non-destructive, high-resolution imaging of dental structures. It allows for the detailed evaluation of root canal systems and offers valuable insights into anatomical complexities that may contribute to treatment failure [13]. Micro-CT provides clinicians and researchers with a powerful tool for non-invasively assessing the quality of endodontic treatment and understanding the mechanisms behind failure [14].

Although previous studies have retrospectively explored reasons for endodontic failure and extraction, including morphological differences between premolars and molars [15], few have utilised micro-CT to conduct a detailed morphometric analysis of extracted teeth selected based on specific clinical criteria. In a related investigation, apical root segments obtained during

periapical surgery were examined using micro-CT and cone-beam computed tomography (CBCT) to assess procedural errors, lesion sizes, and obturation quality [13]. Additionally, the microbiologic, tomographic, and histopathologic characteristics of post-treatment apical periodontitis have been investigated [14].

However, to the best of our knowledge, no study has comprehensively analysed extracted endodontically treated first molars using micro-CT, focusing specifically on both root canal anatomy and filling quality in relation to clinical failure. Therefore, the aim of this study was to investigate the potential factors contributing to endodontic failure in extracted first molars by using micro-CT to assess root canal morphology, filling length, and obturation quality.

Materials and methods

The manuscript of this *in vitro* study has been prepared in accordance with the Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines [16]. All procedures were conducted following the principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Yildirim Beyazıt University Institutional Ethics Committee (protocol number: 2021-37).

Teeth collection process

A total of 348 maxillary and mandibular first molars (MxFMs and MnFMs), that had previously undergone endodontic treatment but subsequently failed and required extraction were collected from the Department of Oral and Maxillofacial Surgery at Yildirim Beyazıt University. The sample collection process was carried out between 19 April and 19 July, 2021. Prior to extraction, patients were informed both verbally and in writing about potential risks and complications, and written informed consent was obtained.

To collect study-specific data not available in digital patient records, a dedicated form was created. Each patient was examined by an endodontist who was blinded to the study objectives but familiar with the eligibility criteria and responsible for completing the form. Extractions were performed by clinicians who were also blinded to the study.

At the end of each working day, extracted endodontically treated first molars were placed in saline-filled plastic containers labelled with the patient's record number. Two endodontists and two periodontists, each with a minimum of five years of clinical experience, independently reviewed the recorded data and radiographic images to determine eligibility. In cases of disagreement, a third specialist was consulted to reach consensus.

Teeth meeting the inclusion criteria were carefully cleaned of any soft tissue attachments using a periodontal

curette and stored in Eppendorf tubes containing 0.1% thymol solution diluted in distilled water. Samples were kept at room temperature in a dark environment throughout the study period.

Inclusion and exclusion criteria

This study included MxPMs and MnPMs extracted from patients without systemic health issues, classified as ASA Physical Status I according to the American Society of Anaesthesiologists Physical Status Classification System. All included teeth had undergone primary conventional root canal treatment, exhibited periodontal health, and had satisfactory coronal restorations.

Periodontal health was assessed through both radiographic and clinical examinations. Radiographic evaluation determined alveolar bone levels, while clinical assessment included probing pocket depth (PPD), bleeding on probing (BOP), and clinical attachment loss (CAL). Periodontal parameters were recorded at six points per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) using a standard periodontal probe (PCP-UNC-156, Hu-Friedy Mfg. Co., LLC, Chicago, IL, USA). Periodontal health was defined as gingival health without periodontitis, characterised by $PPD \leq 3$ mm, absence of BOP, and no gingival inflammation with maintained oral hygiene [17]. Patients with an intact periodontium, without interproximal CAL, and without radiographic bone loss were included.

Coronal restorations were evaluated using the Modified US Public Health Service (USPHS)/Ryge Clinical Evaluation Criteria and Scoring system [18]. Teeth were included if their restorations scored 'A' for marginal adaptation, marginal discolouration, secondary caries, and fractures, and 'A' or 'B' for anatomical form and surface roughness. Postoperative sensitivity and colour matching were not considered in the evaluation.

Although the remaining tooth structure was sufficient for restorative or prosthetic rehabilitation after retreatment, apical resection, or hemisection, these procedures were contraindicated due to extensive periapical lesions or a high risk of detrimental occlusal forces. Periapical radiographs revealed no evidence of fractured instruments or post-core restorations.

The status of primary root canal treatment was verified based on patient records. The included teeth demonstrated wide periapical radiolucency extending beyond the apical third, involving only the mesial root. Diagnoses were either symptomatic apical abscess or asymptomatic apical periodontitis, conditions indicating extraction. Periapical health was evaluated using the Periapical Index (PAI) score. Owing to the COVID-19 pandemic, strict triage was applied; only teeth with PAI scores of 4 (periodontitis with well-defined radiolucency) or 5 (severe

periodontitis with acute flare-up characteristics) were included.

The consequences of tooth extraction were also discussed in detail, and patient questions were addressed carefully. In the context of the pandemic, which is characterised by limited access to advanced dental care, uncertain follow-up opportunities, patient treatment expectations, and resource constraints, these risks were comprehensively discussed. Following a shared decision-making process, verbal and written informed consent was obtained from patients who voluntarily opted for extraction after understanding the alternatives (non-surgical retreatment versus extraction) and associated risks.

Exclusion criteria encompassed dental anomalies, a history of dental trauma, prior endodontic retreatment, apical surgery, mineral trioxide aggregate (MTA) apical plug placement, or perforation repair procedures. Teeth were also excluded if their structural integrity was compromised during extraction, if the status of the primary treatment was unclear, or if the patient had systemic health conditions.

A total of 43 teeth ($n=43$), extracted from 42 patients aged between 18 and 55 years, met the inclusion and exclusion criteria and were included in the study. One patient contributed two extracted teeth.

Micro-CT scanning protocol and analysis

A high-resolution micro-CT system (Bruker SkyScan 1275, Kontich, Belgium) was employed to perform internal and three-dimensional (3D) morphological analyses of the extracted teeth. Prior to scanning, specimens were stored in a 100% humid environment to prevent dehydration, with no mechanical or chemical pretreatment applied.

Scanning parameters were set at 80 kV, 125 μ A, and an isotropic voxel size of 18 μ m. A full 360° rotation with a step angle of 0.2° was performed to capture comprehensive internal and external morphologies. Teeth were stabilised in a sample holder to minimise motion artefacts during scanning.

Raw data were reconstructed using NRecon software (Bruker, v1.7.4.2) employing retrospective filtering algorithms, yielding 16-bit greyscale cross-sectional images. These images were analysed with CTAn software (Bruker, v1.23.0.2) via a semi-automatic method. To enable detailed evaluation of dental structures and root canals, 3D volumetric rendering was performed.

The system was calibrated according to the manufacturer's instructions to ensure scanning accuracy. Beam hardening artefacts, which are commonly caused by high-density structures, such as metallic restorations or fractured instruments, were minimised to enhance image quality.

The micro-CT data obtained were employed to evaluate void percentages within the root canal filling materials (gutta-percha and sealer), root canal configurations, and the presence of untreated extra canals.

Since voids and gaps were indistinguishable in micro-CT images, all unfilled areas within the root canal space were classified as voids. Lateral and accessory canals were excluded from the analysis [19].

The isthmus was categorized according to the following classification:

- *Type I*: Incomplete isthmus
- *Type II*: Complete connection between two canals
- *Type III*: Very short complete connection between two canals
- *Type IV*: Complete or incomplete connection between three or more canals
- *Type V*: Two or three openings without a visible connection

Root canal filling length was evaluated as follows:

- *Ideal*: Filling ending ≤ 2 mm from the apex
- *Short*: Filling ending > 2 mm from the apex
- *Overfilled*: Filling extending > 0 mm beyond the apex

All micro-CT analyses were performed blindly by an experienced operator with 14 years of expertise (M.O.).

Statistical analysis

All statistical analyses were conducted using SPSS version 22 (IBM Corp., Armonk, NY, USA). A total of 43 teeth were assessed based on the presence of extra canals, root canal configuration, isthmus presence and classification, root canal filling length, and the percentage of voids within the root canal filling.

Demographic and morphological characteristics of the sample were summarised using descriptive statistics (n , %, mean $\pm$ SD, median [min–max]). The primary outcome variable, voids within the root canal space (%), was compared between groups using independent-samples t-tests. Normality was assessed with the Shapiro–Wilk test, and homogeneity of variances with Levene's test; where homogeneity was violated, Welch's adjusted t-test was applied. An alpha level of 0.05 was adopted for all analyses, and two-tailed tests were applied. Statistical significance was set at $p < 0.05$.

Results

A total of 43 extracted first molars ($n = 43$; 26 mandibular and 17 maxillary) were analysed using micro-CT. These teeth were obtained from 42 patients, of whom 27 (64.3%) were male and 15 (35.7%) were female. Six patients (14.3%) were aged 18–26, 18 (42.9%) were aged

27–45, and 18 (42.9%) were aged 46–55. One patient contributed two first molars to the study.

Clinical and morphological findings

Table 1 summarises the distribution of the evaluated clinical and micro-CT parameters. Most teeth had either mesio-occlusal/distal or mesio-occluso-distal restorations. In terms of canal morphology, a wide variety of configurations were observed in both MxFMs and MnFMs. Among the extra canals, the middle mesial canal (MMC) was present in 50.0% of MnFMs, while the second mesiobuccal canal (MB2) was identified in 70.6% of MxFMs. However, these extra canals were mostly unfilled.

Isthmus classification and root canal fillings

Isthmus morphology varied significantly between MnFMs and MxFMs. In MnFMs, the most common classification was Type 2 (46.2%), while the isthmus was absent in 26.9%. In contrast, 70.6% of MxFMs exhibited no isthmus at all. The length of root canal filling was classified as ideal in 44.2% of all cases, short in 48.8%, and overfilled in 7.0%. The anatomical and technical features were further visualized and exemplified using micro-CT imaging, as presented in Fig. 1.

Void analysis

The void percentages in the root canal fillings differed significantly between apical and coronal regions. As displayed in Table 2, MnFMs exhibited mean void percentages of 14.5% (apical) and 6.6% (coronal), whereas MxFMs had higher mean values at 16.4% (apical) and 8.0% (coronal). These differences were statistically significant ($p < 0.001$). When grouped by region regardless of tooth type, the mean void percentage in the apical third (15.3%) was significantly greater than that in the coronal third (7.2%) ($p < 0.001$), as detailed in Table 3.

Discussion

This observational in vitro study analysed the mesial roots of MxFMs and MnFMs with endodontic failure after primary, non-surgical root canal treatment. Using micro-CT imaging, parameters such as root canal configuration, presence of extra canals, isthmus types, obturation length, and void percentages were assessed. The study intentionally focused on a narrow clinical subset to reduce confounding variables and enhance internal validity. Although this focus limits generalisability, it was a deliberate methodological choice—not a source of selection bias—allowing for a more precise analysis of endodontic failure mechanisms.

Traditional clinical evaluations rely on periapical radiographs and CBCT. While periapical radiographs offer only two dimensional (2D) imaging, CBCT provides

Table 1 Distribution (%) of clinical and micro-computed tomography parameters in mesial roots of mandibular and maxillary first molars

		Mesial roots of MnFMs		Mesial roots of MxFMs		Total	
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Restoration	Crown	4	15.4	0	0.0	4	9.3
	Mesio-Occlusal/Occluso-Distal	10	38.5	7	41.2	17	39.5
	Mesio-Occluso-Distal	8	30.8	6	35.3	14	32.6
	Occlusal	4	15.4	4	23.5	8	18.6
	Total	26	100.0	17	100.0	43	100.0
Canal configuration	A-Type 2	6	23.1	-	-	6	14.0
	B-Type 4	6	23.1	-	-	6	14.0
	C-Type 8	1	3.8	-	-	1	2.3
	D-Type 1	2	7.7	-	-	2	4.7
	D-Type 2	4	15.4	-	-	4	9.3
	D-Type 3	5	19.2	-	-	5	11.6
	A-Type 2	1	3.8	-	-	1	2.3
	D-Type 1	1	3.8	-	-	1	2.3
	A-Type1	-	-	5	29.4	5	11.6
	A-Type2	-	-	8	47.1	8	18.6
	B-Type4	-	-	3	17.6	3	7.0
	B-Type7	-	-	1	5.9	1	2.3
	Total	26	100.0	17	100.0	43	100.0
Extra canal	Second mesiobuccal canal	-	-	12	70.6	12	27.9
	Middle mesial canal	13	50.0	-	-	13	30.2
	Absent	13	50.0	5	29.4	18	41.9
	Total	26	100.0	17	100.0	43	100.0
Isthmus classification	Type 2	12	46.2	4	23.5	16	37.2
	Type 4	5	19.2	-	-	5	11.6
	Type 1	2	7.7	-	-	2	4.7
	Type 3	-	-	1	5.9	1	2.3
	Absent	7	26.9	12	70.6	19	44.2
	Total	26	100.0	17	100.0	43	100.0
Root canal filling length	Ideal	9	34.6	10	58.8	19	44.2
	Short	15	57.7	6	35.3	21	48.8
	Overfilled	2	7.7	1	5.9	3	7.0
	Total	26	100.0	17	100.0	43	100.0

n = 43 teeth; obtained from 42 patients, one contributed two teeth. MxFMs: Maxillary first molars, MnFMs: Mandibular first molars

3D views, albeit with limitations in visualising fine apical anatomy [20]. In contrast, micro-CT delivers high-resolution, non-destructive, 3D assessments of extracted teeth, offering both quantitative and qualitative data comparable to histological analysis [21, 22]. Additionally, it is superior in detecting procedural errors such as missed canals, zipping, ledge formation, and apical transportation [13], providing critical insights into the causes of endodontic failure in molars.

Fransson et al. have stressed the lack of research on prognostic factors influencing long-term survival of root canal-treated teeth, underlining the significance of tooth restorability and the quality of post-endodontic restoration [6]. Similarly, Zadik et al. found that most extractions following endodontic treatment were due to nonrestorable caries, while treatment failures and complications like fractures or perforations were less frequent

[23]. In contrast, the current study excluded teeth with compromised restorations to isolate endodontic causes of failure. This methodological decision allowed for an accurate evaluation of internal morphology and technical adequacy of the treatment.

Previous studies assessing reasons for extraction often lacked strict inclusion criteria, analysing any root canal-treated teeth planned for removal [23, 24]. Our study applied tighter selection standards—excluding systemic health issues, unclear treatment status, or compromised tooth structure—thus allowing a focused analysis of internal anatomical features related to failure. This rigorous exclusion process is particularly important, as molars are frequently extracted due to their complex anatomy and increased susceptibility to technical complications [3, 25]. As primary treatments generally yield higher

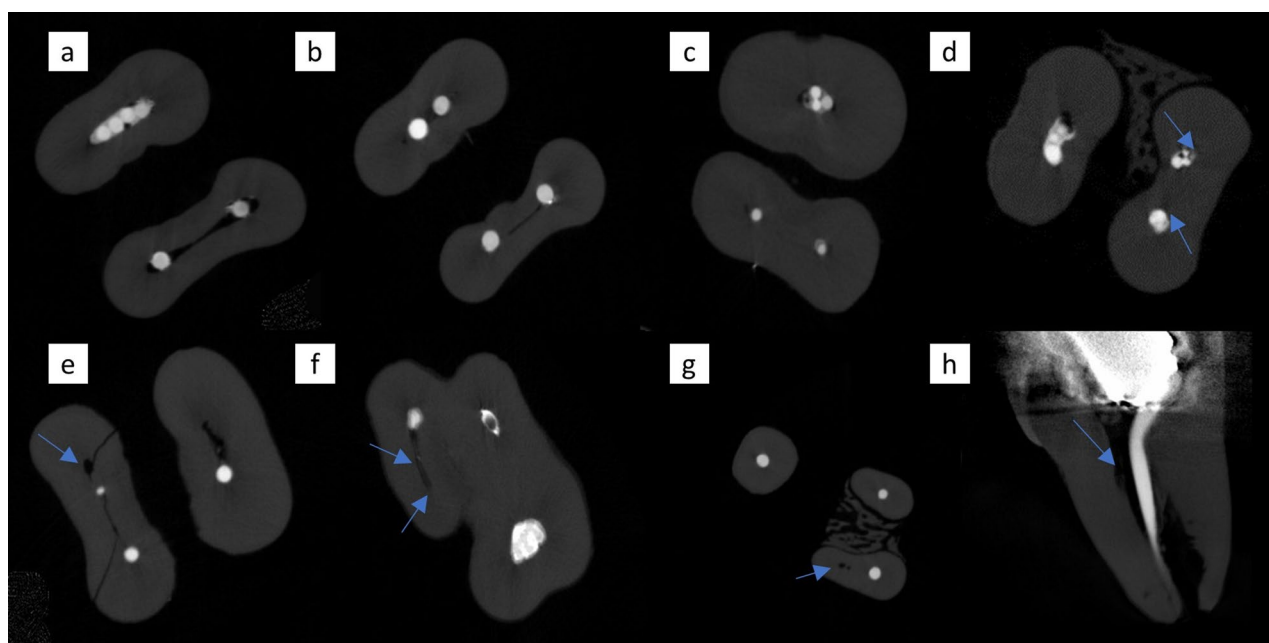


Fig. 1 Representative axial and frontal micro-computed tomography images illustrating the evaluated parameters. **a–c** Depict isthmus morphology: **a** Type 2 isthmus (complete connection between two canals), **b** Type 1 isthmus (incomplete), and **c** absence of an isthmus. **d** Arrows indicate voids in the mesiobuccal and mesiolingual root canal fillings of a mandibular first molar. **e** The middle mesial canal (MMC) of a mandibular molar is obturated, while the mesiobuccal canal remains untreated. **f, g** Missed second mesiobuccal canals (MB2) in maxillary first molars. **h** Extensive voids are observed in the root canal filling of the mesiobuccal canal in a mandibular molar

Table 2 Percentage of voids (%) in root canal fillings according to tooth location

								t test
		<i>n</i>	Mean ± Sd	Median	Minimum	Maximum	<i>t</i>	<i>p</i>
MnFMs	Apical	26	14.5 ± 4.2	13.8	6.8	22.6	8.5	0.0001
	Coronal	26	6.6 ± 2.3	6.6	3.4	10.3		
	Total	52	10.6 ± 5.2	10.2	3.4	22.6		
MxFMs	Apical	17	16.4 ± 4.8	17.4	6.7	24.3	6.4	0.0001
	Coronal	17	8.0 ± 2.4	8.2	3.6	10.7		
	Total	34	12.2 ± 5.6	10.5	3.6	24.3		

n = 43 teeth. Data were analysed using an independent samples t-test. Sd: Standard deviation, MxFMs: Maxillary first molars, MnFMs: Mandibular first molars, *p* < 0.05 is statistically significant

Table 3 Percentage of voids (%) in root canal fillings according to root regions

	<i>n</i>	Mean	Median	Minimum	Maximum	Sd	<i>t</i>	<i>p</i>
Apical	43	15.3	15.4	6.7	24.3	4.4	10.5	0.0001
Coronal	43	7.2	7.3	3.4	10.7	2.4		
Total	86	11.2	10.4	3.4	24.3	5.4		

n = 43 teeth. Data were analysed using an independent samples t-test. Sd: Standard deviation, *p* < 0.05 is statistically significant

success rates than retreatments [6], only teeth with primary treatment were included.

The prevalence of MB2 canals in MxFMs has been reported to be approximately 69.6% [26]. Failure to detect and treat MB2 canal is a well-established cause of endodontic failure [27, 28]. In this study, MB2 canals were present in 70.6% of MxFMs, consistent with the literature. The high failure rate in these teeth may reflect the inherent difficulty of locating and instrumenting MB2 canals, which are often obscured by secondary dentin

and located beneath the dentinal wall [27]. Canal configuration findings in the current study (Type 2: 47.1%, Type 1: 29.4%, Type 4: 17.6%, and Type 7: 5.9%) align partially with previous studies [29–31], though differences may result from variations in methodology and study population.

Similarly, the presence of MMC canals in MnFMs is a clinically relevant anatomical variation. Reported prevalence varies widely depending on the detection method—from 0.0% to over 50.0% [32, 33]. In our study, MMC

canals were detected in 50.0% of MnFMs, a high rate that may stem from the use of micro-CT and the specific inclusion criteria of endodontically failed teeth. Isthmus structures were also highly prevalent, observed in 73.1% of MnFMs and 29.4% of MxFMs, predominantly as Type 2. These findings underscore the complexity of molar anatomy and its potential to contribute to treatment failure when left unaddressed.

The inability to clean and fill untreated or missed canals remains a key factor in persistent endodontic infections [28, 34]. Karabucak et al. found a 4.5-fold increase in apical lesion risk in teeth with missed canals [34]. This finding is consistent with our observation that none of the MB2 or MMC canals were treated. This study reinforces the clinical importance of thoroughly identifying and managing all canal systems.

The isthmus, which is often challenging to detect and clean, may harbour residual debris and bacteria that contribute to failure [35, 36]. Arx et al. demonstrated the presence of unfilled isthmuses in molars requiring apical surgery, highlighting the difficulty of thorough debridement with conventional techniques [37]. Our micro-CT findings further emphasise the importance of recognising and addressing isthmuses during root canal therapy.

In terms of technical factors, root canal filling length was classified as ideal in only 34.6% of MnFMs and 58.8% of MxFMs. Previous studies have shown that fillings ending within 0–1 mm of the apex are associated with better outcomes [38, 39]. Voids within the obturation material, particularly in the apical region, were more frequent than in the coronal portion. This is consistent with Machado et al., who reported a correlation between apical voids and symptomatic apical periodontitis [13]. Such voids provide a favourable environment for bacterial persistence, undermining treatment success.

The observed apical void percentages, significantly higher than coronal ones in both MxFMs and MnFMs ($p < 0.001$), highlight the difficulty in achieving dense and homogeneous obturation in apical regions. These technical shortcomings likely contributed to treatment failures in the study sample, emphasising the importance of high-quality obturation techniques.

Despite the valuable insights, the study has limitations. Data regarding sealer type, irrigation protocols, operator experience, number of visits, and intracanal medicaments were unavailable. The strict inclusion criteria—while enhancing internal validity—limit the generalisability of the findings. Furthermore, the relatively small sample size necessitates caution in extrapolating results to the broader population.

Nonetheless, this study demonstrates the critical role of advanced imaging technologies like micro-CT in understanding root canal morphology and endodontic failure. Future research should aim to improve canal

detection methods, enhance instrumentation protocols, and refine obturation techniques. Clinicians are encouraged to adhere to evidence-based protocols and incorporate magnification and imaging technologies to minimise treatment failures and improve long-term outcomes.

Conclusion

This study demonstrated that missed anatomical variations such as MB2 and MMC canals, the presence of isthmuses, and inadequate root canal obturation—particularly in the apical region—were common findings in endodontically failed molars. Micro-CT analysis allowed a detailed evaluation of these internal factors, emphasising the critical importance of thorough cleaning, shaping, and filling of the entire root canal system. Recognising and managing complex root anatomy remains essential for improving the long-term success of root canal treatments.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12903-025-07301-7>.

Supplementary Material 1.

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The authors deny any conflict of interest related to the present study.

Authors' contributions

N.B.K. conceptualisation (equal), investigation (equal), writing – review and editing (equal), funding acquisition (lead); E.O.T. formal analysis (equal), writing – original draft preparation (lead); G.U. conceptualisation (equal), writing – review and editing (equal); A.M.T. conceptualisation (equal), investigation (equal); M.G. conceptualisation (equal), supervision (lead), validation (lead); U.C.K. investigation (equal); M.O. formal analysis (equal), visualisation (lead), data curation (lead).

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee of Yıldırım Beyazıt University (Protocol No: 2021-37). All procedures were conducted in accordance with the ethical standards outlined in the Declaration of Helsinki by the World Medical Association (WMA). Every human participant provided their informed consent prior to participation in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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