

Comparative Analysis of Root Canal Morphology in Human Mandibular Permanent Incisors using Decalcification Technique and Periapical Radiographs: An In-vitro Study

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ABSTRACT

Introduction: Understanding the intricate root canal system of mandibular incisors is crucial for successful root canal therapy. Variations in canal anatomy pose challenges in diagnosis and treatment planning, emphasising the need for comprehensive evaluation methods.

Aim: To evaluate and assess the root canal configuration of permanent mandibular incisors based on Vertucci's classification using periapical radiographs and correlating the same with the gold standard decalcification and dye-penetration technique.

Materials and Methods: The current in-vitro study was carried out from December 2018 to March 2019 in the Department of Conservative Dentistry and Endodontics, Dr. R. Ahmed Dental College and Hospital, Kolkata, West Bengal, India. It analysed the root canal morphology of mandibular central and lateral incisors using radiographic examination and decalcification-dye penetration evaluation. Data from 50 central and 50 lateral incisors (100 mandibular incisors) were procured and were assessed and subsequently analysed statistically using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) Statistics for Windows, Version 27.0 (Armonk, NY: IBM Corp). The study outcome included assessing the various canal types and configurations, types of canals

(straight/curved), and the position of the apical foramen (labial/lingual) by both techniques. The existence of agreement was also tested using statistical analysis by the kappa (κ) coefficient between the two techniques with an alpha level of 5%.

Results: The radiological findings of mandibular central incisors revealed a predominant presence of Type-I canals, constituting 66 (66%) cases, according to Vertucci's classification. Decalcification-dye penetration evaluation of central incisors showed a similar trend, with Type-I canals exhibiting a significantly higher percentage 35 (70%) compared to other canal types ($p < 0.001$). Lateral incisors exhibited similar trends in both radiological findings and decalcification-dye penetration. Type-I canals were significantly more prevalent 29 (58%) compared to other types ($p < 0.001$). Evaluation with the decalcification-dye penetration technique further supported this, with Type-I canals showing a higher percentage 27 (54%) compared to other types ($p < 0.001$). A moderate amount of agreement was detected between the two modalities concerning canal configuration ($\kappa = 0.46$, $p = 0.049$) and position of the apical foramen ($\kappa = 0.43$, $p < 0.001$), while an almost perfect agreement was present on canal curvature ($\kappa = 0.85$, $p < 0.001$).

Conclusion: These results collectively suggest a similarity in the radiological characteristics and decalcification-dye penetration patterns between central and lateral incisors.

Keywords: Anatomy, Canal configuration, Mandibular incisors

INTRODUCTION

The successful execution of root canal therapy in mandibular incisors relies on a deep understanding of their intricate root canal system [1]. This complex task requires considering various factors such as tooth morphology, precise radiographic interpretation, accurate access preparation, and thorough exploration of the internal structure [2,3]. The main goal is to shape, clean, and fill all pulp spaces effectively. An accurate diagnosis and comprehensive root canal filling are essential.

Endodontics involves dealing with diverse tooth anatomies, including extra root canals, lateral canals, and intricate apical structures. Detecting these variations can be challenging, especially in mandibular incisors due to their unique orientation and elusive access points. Studies have investigated anatomical variations in teeth, particularly mandibular incisors, uncovering differences in the reported prevalence of additional canals [2-5]. Discrepancies arise from variations in study design, techniques for canal identification, and racial differences in dental anatomy. Understanding dental anatomy extends to exploring genetic influences on anatomical differences, contributing to knowledge in both endodontics and anthropology.

Even seemingly "simple" teeth like single-rooted incisors can exhibit complex canal systems. Weine's 1969 classification categorised root canal systems into four types, but Vertucci's eight-type classification is considered more practical [5].

Various techniques, including periapical radiographs, decalcification, dye penetration, confocal microscopy, stereomicroscopy, and advanced imaging like Cone Beam Computed Tomography (CBCT) and micro-Computed Tomography (μ -CT), are used to study root canal anatomy [6]. Periapical radiographs were employed in the current study as it's the most commonly used diagnostic method used at the chairside, as other methods cannot be used clinically, except CBCT, which is recommended to be used in only specific cases [7].

The present study aimed to evaluate the root canal morphology and configurations in human mandibular permanent incisors using Vertucci's classification and decalcification technique, highlighting the lack of previous research on the correlation between Intraoral Periapical Radiographs (IOPAR) and canal configuration. The null hypothesis of the following study was that there was no agreement between the findings of IOPAR and the decalcification-dye penetration technique in assessing root canal morphology in mandibular incisors.

MATERIALS AND METHODS

The current in-vitro study was carried out from December 2018 to March 2019, in the Department of Conservative Dentistry and Endodontics after obtaining approval from the Institutional Ethics Committee of Dr. R. Ahmed Dental College and Hospital, Kolkata, West Bengal, India.

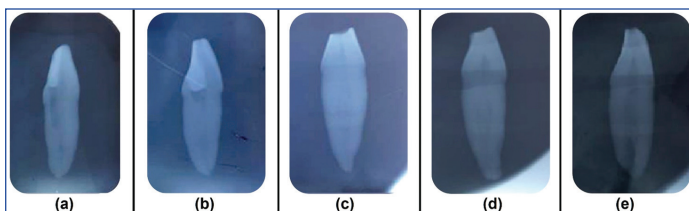
Sample size calculation: A minimum sample size of 96 specimens, with 48 specimens per type of tooth (central incisors and lateral incisors, respectively), was established using G*Power Software version 3.1.9.7. The sample size calculation utilised a chi-square goodness-of-fit test model with an effect size of 0.5, an alpha error of 0.05, a power of 80%, and a two-tailed significance level (α) of 0.05. The effect size was based on a prior study [8]. However, the decision was made to conduct the study on 50 central and 50 lateral incisors each.

Study Procedure

A total of one hundred (N=100) freshly extracted mandibular incisors (fifty central and 50 lateral incisors) for orthodontic purposes were obtained from the Department of Oral and Maxillofacial Surgery of the Dental College and Hospital from the patients visiting the Dental College. The specimens were selected based on having an intact crown structure, fully-formed apex, and being free of caries, restorations, and cracks. The samples were stored in a 5.25% Sodium Hypochlorite (NaOCl) solution (Prime Dental, Thane, India) for disinfection for 24 hours before being placed in an ultrasonic bath for 30 minutes to eliminate any soft tissue remnants.

The specimens were then evaluated using IOPAR and the decalcification-dye penetration technique to assess the various canal types and configurations, types of canals (straight/curved), and the position of the apical foramen (labial/lingual). Additionally, the findings of the two modalities were correlated for the canal configurations.

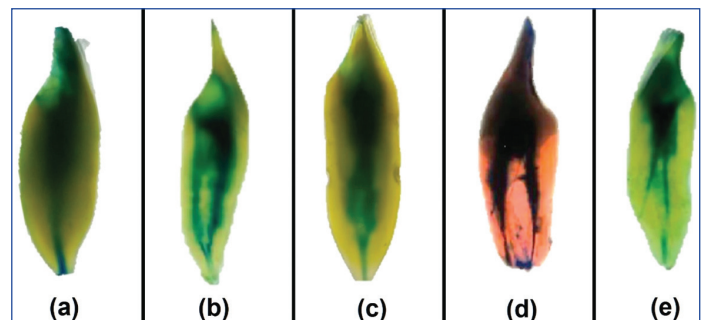
IOPAR evaluation: The tooth samples were stabilised with modeling wax on the E-speed film measuring 30.5x40.5 mm (Carestream Health Inc., Rochester, NY, USA 14608). Images were obtained on both labio-lingual and proximal aspects using the bisector technique and standard horizontal angulation, with the object-to-X-ray distance kept at 16 mm. The films were exposed for 3.2 seconds at 70 Kvp and 8 mA. After removing the teeth from the radiography film, they were remounted in a mesiodistal orientation. The film was exposed once more with the object source distance set at 16mm. Each radiograph was manually developed, fixed, and dried before being placed on a hand-held platform [9]. The proximal view was utilised to find the position of the apical foramen, while the bucco-lingual view was used to assess the root canal curvature. The radiographic images obtained for the various canal configurations is illustrated in [Table/Fig-1].



[Table/Fig-1]: Canal configurations according to Vertucci's classification assessed by intraoral periapical radiograph-(a) Type I; (b) Type II; (c) Type III; (d) Type IV; and (e) Type V [1].

Decalcification-dye penetration technique: A conservative access cavity preparation was performed using no. 2-round TC burs (SS White Dental, New Jersey, USA), and the outline was widened labiolingually and smoothed with an Endo-Z bur (Dentsply Maillefer, Germany) mounted on a high-speed handpiece (NSK, Nakanishi, Japan). Following this, the canal was profusely irrigated with 5.25% NaOCl and immersed in the same solution overnight. Instrumentation was avoided to prevent manipulation of the canal morphology. Subsequently, the teeth were rinsed under running

water for 20 minutes to remove all debris from the root canals, followed by 24 hours of storage for complete drying, which is necessary to facilitate the clearing procedure. The samples were then placed in test tubes containing 5% Nitric acid (Nice Chemical Pvt., Ltd., Kochi, India) for three consecutive days, with periodic replenishment and intermittent agitation to allow for uniform decalcification. Following this process, the samples were thoroughly washed with water to get rid of any acidic residues. They were then placed in increasing concentrations of ethyl alcohol (Changshu Hongsheng Fine Chemical Co. Ltd., Changshu, China) for dehydration, with each concentration (70%, 80%, and finally 90%) being applied for 24 hours. Subsequently, the samples were immersed in a 2% methyl salicylate solution (Merck Specialties Pvt., Ltd., Mumbai, India) to enhance complete clarity for visualising minor anatomical variations. Methylene blue dye (Merck Life Science Pvt., Ltd., Mumbai, India) was then injected using a 27-gauge Luer-lock syringe, while maintaining negative pressure with a medium-volume suction for proper dispersion of the dye. After this, the dye-penetrated specimens were observed under the Dental operating microscope (HS-Moller-Wedel International, Switzerland) at 4X magnification. An evaluation was then carried out regarding the root canal morphology based on Vertucci's classification [Table/Fig-2] [1].



[Table/Fig-2]: Canal configurations according to Vertucci's classification assessed by decalcification and dye penetration technique-(a) Type I; (b) Type II; (c) Type III; (d) Type IV; and (e) Type V.

STATISTICAL ANALYSIS

Tabulated observations were then subjected to statistical analysis using IBM SPSS Statistics for Windows, Version 27.0 (Armonk, NY: IBM Corp). Descriptive statistics were used to report the categorical variables in frequencies and percentages, and they were tested using the Chi-square test of proportions. The level of agreement between the two modalities was assessed using the kappa (κ) coefficient. The statistical significance level was set at 5% ($p < 0.05$).

RESULTS

The radiological findings of central incisors revealed a predominance of Type-I canals {37 (74%)}, followed by Type-II {9 (18%)}, Type-III {3 (6%)}, and Type-IV {1 (2%)} canals, according to the Vertucci classification [Table/Fig-3]. The test of proportion indicated a statistically significant difference, with Type-I canals being significantly more prevalent than other types ($p < 0.001$). Decalcification-dye penetration evaluation of central incisors showed a similar trend, with Type-I canals exhibiting a significantly higher percentage {35 (70%)} compared to other canal types ($p < 0.001$).

Lateral incisors exhibited similar tendencies in both radiological findings and decalcification-dye penetration. Type-I canals were significantly more prevalent {29 (58%)} compared to other types ($p < 0.001$). The decalcification-dye penetration evaluation further supported this, with Type-I canals showing a higher percentage {27 (54%)} compared to other types ($p < 0.001$).

Cumulatively, mandibular incisors displayed a predominant presence of Type-I canals {66 (66%)}, which was higher than other morphologic types ($p < 0.001$). The cross-tabulated data has been depicted in [Table/Fig-4], which suggested a moderate agreement

Teeth	Vertucci's type	Radiological findings	Decalcification-dye penetration evaluation
Central incisors (n=50)	I	37 (74%)	35 (70%)
	II	9 (18%)	10 (20%)
	III	3 (6%)	1 (2%)
	IV	1 (2%)	1 (2%)
	V	0	3 (6%)
Lateral incisors (n=50)	I	29 (58%)	27 (54%)
	II	13 (26%)	17 (34%)
	III	2 (4%)	2 (4%)
	IV	5 (10%)	4 (8%)
	V	1(2%)	0
Total (N=100)	I	66 (66%)	62 (62%)
	II	22 (22%)	27 (27%)
	III	5 (5%)	3 (3%)
	IV	6 (6%)	5 (5%)
	V	1 (1%)	3 (3%)

[Table/Fig-3]: Distribution of study samples according to findings of root canal morphologic variants according to Vertucci's system by periapical radiographs and decalcification-dye penetration technique in permanent mandibular incisors.

($\kappa=0.46$, $p=0.049$) between the two methods regarding canal configurations, suggesting that the distribution of canal configuration was not dependent on the type of modality, thereby implying that IOPAR evaluated canal configurations in mandibular incisors quite similarly to the decalcification-dye penetration technique, thereby rejecting the null hypothesis.

Radiological findings	Decalcification-dye penetration evaluation					Total	
	Vertucci's Types	I	II	III	IV		V
I		40	16	3	5	2	66
Row%		60.6	24.2	4.5	7.6	3	100
Col%		64.5	59.3	100	100	66.7	66
II		15	7	0	0	0	22
Row%		68.2	31.8	0	0	0	100
Col%		24.2	25.9	0	0	0	22
III		4	0	0	0	1	5
Row%		80	0	0	0	20	100
Col%		6.5	0	0	0	33.3	5
IV		3	3	0	0	0	6
Row%		50	50	0	0	0	100
Col%		4.8	11.1	0	0	0	6
V		0	1	0	0	0	1
Row%		0	100	0	0	0	100
Col%		0	3.7	0	0	0	1
Total		62	27	3	5	3	100
Row%		62	27	3	5	3	100
Col%		100	100	100	100	100	100

[Table/Fig-4]: Cross-tabulation between the findings of root canal morphologic variants according to Vertucci's system by periapical radiographs and decalcification-dye penetration technique in permanent mandibular incisors.

Row%: calculates the percentage of each canal type according to Decalcification-dye penetration evaluation; Column%: calculates the percentage within each canal type according to Radiologic evaluation

Additionally, the radiological evaluation revealed that 90 (90%) of the study samples had a straight canal opening with an apical location of apical foramen, the proportion of which was significantly higher than curved canals and a labial or lingual location of the foramen, respectively ($p<0.001$). The decalcification-dye penetration evaluation revealed a significant prevalence of straighter canals 87 (87%) and an apical location of the apical foramen 71 (71%) in mandibular incisors ($p<0.001$). An almost perfect agreement was

observed between the two modalities concerning the curvature ($\kappa=0.85$, $p<0.001$), while there was a moderate agreement with respect to the position of the foramen ($\kappa=0.43$, $p<0.001$). The distribution of the study samples for both methods according to the curvature and apical foramen location, respectively is depicted in [Table/Fig-5,6].

Radiological findings	Decalcification-dye penetration evaluation		Total
	Curved	Straight	
Curved	1	9	10
Row%	10	90	100
Col%	7.7	10.3	10
Straight	12	78	90
Row%	13.3	86.7	100
Col%	92.3	89.7	90
Total	13	87	100
Row%	13	87	100
Col%	100	100	100

[Table/Fig-5]: Crosstabulation between the findings of root canal morphologic variants according to type of curvature (straight/curved) by periapical radiographs and decalcification-dye penetration technique in permanent mandibular incisors. Row%: calculates the percentage of curvature type according to Decalcification-dye penetration evaluation; Column%: calculates the percentage of curvature type according to Radiologic evaluation

Radiological findings	Decalcification-dye penetration evaluation		Total
	Apex	Labially/Lingually	
Apex	63	27	90
Row%	70	30	100
Col%	88.7	93.1	90
Labially/Lingually	8	2	10
Row%	80	20	100
Col%	11.3	6.9	10
Total	71	29	100
Row%	71	29	100
Col%	100	100	100

[Table/Fig-6]: Cross-tabulation between the findings of root canal morphologic variants according to location of apical foramen (apex/labially or lingually) by periapical radiographs and decalcification-dye penetration technique in permanent mandibular incisors.

Row%: calculates the percentage of foramen location type according to Decalcification-dye penetration evaluation; Column%: calculates the percentage of foramen location type according to radiologic evaluation

DISCUSSION

The present in-vitro study aimed to assess the root canal morphology of human mandibular permanent incisors using a combination of decalcification techniques and periapical radiographs. This comprehensive approach allowed for a detailed understanding of the canal anatomy, including its classification according to Vertucci, where Type-I was found to be the most prevalent by both techniques, the predominant presence of straighter canals, and the primary opening of the foramen being at the apex. The study aimed to correlate the findings of IOPAR in detecting the canal morphology with the gold standard method of decalcification and dye penetration. A moderate to almost perfect agreement was observed between the two techniques for the various study parameters, thus rejecting the null hypothesis.

In clinical practice, Intraoral Periapical Radiography (IOPAR) is a fundamental modality for understanding root canal systems. In the present study, extracted teeth were radiographed buccolingually and mesiodistally. This radiographic evaluation was followed by decalcification and dye penetration to reveal the root canal anatomy and compare it with the information obtained through IOPA Radiographs.

The decalcification-dye penetration technique used in the present study, compared to other clearing techniques, is non-destructive, non-invasive, more accurate, and provides finer details of the root canal system in its original three-dimensional form while maintaining the relationship of canals. This technique, originally described by Brain EB in 1966 and modified by Robertson D et al., in 1980, is a valuable tool for studying root canal anatomy [10,11].

In the analysis of lower central and lateral incisors, the study found that the majority of central incisors radiologically exhibited Vertucci's type I canal, indicating a single canal, with a significant percentage of 37 (74%) compared to other types. This finding aligns with Pineda F and Kuttler Y's study in 1972 [12]. However, Sert S and Bayirli GS, reported a lower percentage (32.5%) of type I canals in central incisors in their study [13].

Moreover, the canals were predominantly straight {92 (92%)}, contrasting with the results of Pineda and Kuttler's study in 1972, where a lower percentage of straight canals (63.9%) was observed [13]. The location of apical foramina at the apex was a predominant feature {66 (66%)}, diverging from Pineda F and Kuttler Y's findings in 1972 (83%) [12].

In the decalcification and dye penetration technique, a similar trend was observed, with a significant percentage of type I canals {70 (70%)} in central incisors, consistent with studies by Rahimi S et al., (61.71%) and Peiris R, (66%) [14,15]. The majority of canals were straight {88 (88%)}, and apical foramina were primarily located at the apex {70 (70%)}, aligning with the radiographic findings.

For lateral incisors, the radiographic analysis revealed a high percentage of type I canals {29 (58%)}, in agreement with Almohaimede A et al., (56.95%) study, however in a lower percentage than from Pineda F and Kuttler Y's findings (76.2%) [8,12]. The canals were mostly straight (88%), and apical foramina were commonly found at the apex (70%).

The decalcification technique in lateral incisors also showed a significant percentage of type I canals {27 (54%)}, consistent with the radiographic findings. The canals were predominantly straight {91 (91%)}, and the apical foramina were mainly located at the apex {72 (72%)}.

In the analysis of all mandibular incisors, the radiographic assessment revealed a substantial percentage of type I canals {66 (66%)}, consistent with the findings of Tang Y et al., (77.4%) [16]. The canals were mostly straight {90 (90%)}, and the apical foramina were predominantly located at the apex {90 (90%)}, matching the study by Ch U et al., (98%) [17].

Overall, the results of this in-vitro study provide valuable insights into the root canal morphology of human mandibular permanent incisors, emphasising the importance of utilising both radiographic and decalcification techniques for a comprehensive understanding. The variations observed in canal types, shapes, and apical foramen positions contribute valuable information for clinicians performing root canal therapy.

The in-depth analysis of mandibular incisors, using both radiographic and decalcification techniques, revealed significant findings that contribute to our understanding of root canal anatomy. In the decalcification and dye penetration technique, the majority of mandibular incisors exhibited Vertucci's type I canal {62 (62%)}, aligning with similar studies by Boruah LC et al., (63.75%) [18]. The canals were predominantly straight (87%), and the apical foramina were primarily located at the apex (71%), consistent with Aung NM's findings (70%) [19]. However, diverging results were observed in studies by Boruah LC and Bhuyan AC, (47.25%), indicating varied percentages of straight canals and apical foramina located at the apex [18].

Comparative analyses between central and lateral incisors revealed no significant differences in the type of canal according to Vertucci's classification, the type of canal curvature, and the location of apical foramina. Both radiological and decalcification techniques yielded comparable results for central and lateral incisors.

Further comparisons between radiological and decalcification techniques for mandibular incisors also demonstrated no significant differences in the type of canal, type of canal curvature, and location of apical foramina. This suggests that the evaluation criteria applied to root canal anatomy, including Vertucci's classification, canal curvature, and apical foramina location, remain consistent across both imaging modalities. The results aligned with the results of Assadian H et al., who also reported a moderate degree of agreement between radiographic techniques and sectioning [20]. The current study sheds light on the intricate root canal anatomy of mandibular permanent incisors. Through the use of the decalcification technique and periapical radiographs, the authors have gained valuable insights into the number and types of root canal morphology present in these teeth. However, it cannot be disregarded that only 47% of the findings were in harmony with the decalcification technique (40 in Vertucci Type I and 7 in Vertucci Type II). Nevertheless, the present study findings highlight the complexity and variability of dental anatomy, highlighting the importance of thorough examination and treatment planning in endodontic practice. Moving forward, this knowledge can aid clinicians in achieving more successful outcomes in root canal therapy in adjunct to being aware of variant canal anatomies, ultimately improving patient care and outcomes, as it should be noted that CBCT cannot be taken routinely in all non-surgical endodontic treatment cases. Moreover, taking radiographs at various angulations may help in better assessment of the anatomy and planning the treatment accordingly. However, further research in this area with more advanced techniques would be required to refine the understanding of dental anatomy and validate the results of the current study.

Limitation(s)

The current study employed a conventional radiographic technique, which is subject to processing errors, and considered the decalcification and dye penetration technique as the gold standard, which is also technique-sensitive as an incorrect technique can prevent proper dye penetration, leading to diagnostic errors. Further research using digital radiography and advanced techniques such as μ -CT can be employed to validate the results of the present study.

CONCLUSION(S)

Notwithstanding the limitations of the present study, it was found that type I canals were more prevalent in radiologic images than in the decalcification technique. This discrepancy may result from potential confusion in radiographic images. The decalcification technique offers a more detailed examination, revealing a greater incidence of other root canal types with complexities. The decalcification technique also demonstrated a slightly higher incidence of curved canals compared to radiologic methods, underscoring its efficacy. Similarly, it revealed a higher incidence of labially/lingually positioned apical foramina compared to radiologic methods. In the current study, a moderate amount of agreement was detected between the two modalities concerning canal configuration and the position of the apical foramen, while an almost perfect agreement was present on canal curvature. Overall, the present study emphasises the importance of choosing appropriate methodologies for accurate assessments of root canal morphology in Endodontics.

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