Original Article

Prevalence and morphological analysis of isthmuses in mandibular molars of the Indian population: A micro-computed tomographic study

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Abstract

Objectives: This study evaluated the prevalence, configurations, and correlation of isthmuses at coronal, middle, and apical root 3rd in mandibular molars of the Indian population using micro-computed tomography (μCT).

Materials and Methods: One hundred and five permanent mandibular molar teeth were scanned under μCT. The axial sections were analyzed at the coronal, middle, and apical thirds of the root for isthmus types and classified according to Hsu and Kim's classification. Descriptive statistics for each isthmus type were calculated. The correlations between the apical, middle, and coronal thirds of the root were determined using Pearson's correlation coefficient.

Results: Type IV isthmus was the most common in the coronal third of the mesial root of mandibular 1st molar (42.9%), while Type V was prevalent in the coronal third of the mesial root of 2nd molar (42.9%). Type I isthmus was found to be highly prevalent in the middle 3rd (71.4%) and apical 3rd (61.9%) of mesial roots of 1st molars, and in the middle 3rd (71.4%) and apical 3rd (42.9%) of mesial roots of 2nd molars. Type V isthmus was the most prevalent in all the thirds of the distal roots of both 1st and 2nd molars, ranging from 40% to 50%. Furthermore, a strong correlation of 0.965 ($P < 0.01$) was found between the isthmuses in the apical and middle thirds of roots.

Conclusion: There are variations in the prevalence and type of isthmuses across different sections of the root, including the presence of atypical isthmuses. Micro-CT with high-resolution imaging and three-dimensional reconstruction is crucial for investigating root canal morphology. Clinicians could benefit from considering demographic characteristics to better predict the presence of isthmus variations.

Clinical Relevance: The isthmus configurations and frequency differ at each section of mandibular teeth.

Keywords: Atypical isthmus; mandibular molars; micro-computed tomography; root canal morphology

INTRODUCTION

It is generally accepted that a significant cause of endodontic failure is the inability to locate, debride, shape, and obturate the canals in three dimensions (3D). The internal anatomy of root canals presents many challenges during cleaning and shaping due to variations from normal morphology.[1] It includes isthmuses, accessory canals, additional canals, and apical deltas that are inaccessible during biomechanical preparation. Among these, isthmuses are found to be one of the common causes of treatment failure as they harbor necrotic tissue, bacteria, and their biofilms, and accumulated hard tissue debris (AHTD) produced during root canal instrumentation.[2] Kim *et al*. investigated the effects of an isthmus on the success rate of surgically treated molars. They found that the cumulative survival rate after surgery was 61.5% and 87.4% for 4 years when an isthmus was present and absent, respectively.[3]

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An isthmus, also called corridor, lateral connection, or transverse anastomosis, is an anatomical variant found in root canals and is defined as a narrow, ribbon-shaped communication between two root canals that contain pulp tissue or pulp-derived tissue. Isthmuses can form in the roots of teeth with C-shaped canals, compressed morphology, partial fusion, or nonfusion.[4] Previous studies have been attempted to identify this morphology in different populations, and the incidence of isthmus in the mesial root of the mandibular 1st molars range from 76% to 100%.[5] This wide variation in the prevalence is attributed to ethnic differences.[6,7]

Several techniques that have been used to analyze isthmus patterns in root canals include radiography,[4] root end resection,[5,8] cross sectioning, staining, and clearing,[9] scanning electron microscopy,[10] and stereomicroscopy. However, these conventional techniques were destructive and provided limited two-dimensional information.[5] In recent years, nondestructive imaging modalities such as cone-beam computed tomography (CBCT) and micro-computed tomography (μCT) have been used to investigate the complex morphologies of isthmuses in 3D.[11,12] μCT offers superior resolution compared to CBCT, as it allows for the use of smaller voxel size, enabling the visualisation of fine anatomic structures.[13]

The incidence of various types of isthmuses reported in previous studies has demonstrated significant variability, indicating the influence of factors such as population demographics, study methodology, and root-level considerations.[7,14,15] Current knowledge on the prevalence of isthmus and its variations in the Indian population is sparse, with few studies conducted using either destructive techniques or low-resolution imaging methods.[16] Thus, it is necessary to obtain a comprehensive understanding of this anatomic feature through nondestructive techniques with high resolution. To the best of our knowledge, this is the first study to evaluate the prevalence of isthmus patterns in mandibular molars of the Indian population using μCT.

MATERIALS AND METHODS

The study was conducted in the Department of Conservative Dentistry and Endodontics, and the Institutional Ethics Committee (IECJNMC/507) approved the protocol.

Teeth selection and preparation

A total of 105 extracted permanent mandibular molar teeth were collected. Teeth with caries, root fractures, resorptive defects, restorations, or previous endodontic treatment were excluded. The teeth were stored in normal saline solution and cleaned in 5.25% sodium hypochlorite solution for 1 h to remove tissue remnants and debris. The remaining periodontal tissue and calculus were removed with the ultrasonic instrument. Based on the crown morphology, 63 teeth were identified as mandibular 1st molars and 42 as mandibular 2nd molars.

Micro-computed tomography specifications

The tooth specimens were scanned in a desktop microcomputed tomographic unit (μCT-50; Scanco Medical, Bassersdorf, Switzerland) with the isotropic resolution set at 10 μm. The μCT machine was operated with X-ray settings at 90 kV and 200 μA and 600 ms with 0.1 mm aluminum filter at 360° rotation and a 0.1° rotational step. After processing, a series of tomographic images with approximately 2046 × 2046 voxels was obtained for each specimen.

Image analysis

The mandibular molars were analyzed for isthmus and categorized according to Hsu and Kim classification. The Data viewer μCT Tomography v6.4-2 (Scano Medical) was used for analysis. An independent observer analyzed the entire μCT images, and the analysis and interpretation were confirmed by two other observers. The first slice in each stack of images was located below the cementoenamel junction, and the last slice was considered at the root apex. The mesial and distal roots were divided into; coronal third, middle third, and apical third. Approximately 500–600 slices were chosen under each group and analyzed.

Hsu and Kim classification

The mandibular molars were analyzed at coronal, middle, and apical thirds of the root, as previously described. The isthmus was classified into different types following Hsu and Kim classification [Figure 1].[5] Type I, either two or three canals with no notable communication; Type II, definite connection between the two main canals; Type III, definite connection existing in three canals; Type IV canals extends to the isthmus area; and Type V is identified as a true connection or corridor throughout the section. In addition, variants that did not confirm the above classification were termed as atypical.

Statistical analysis

Descriptive statistics, including frequencies, were calculated for each type of isthmus. Pearson's correlation coefficient was used to assess the correlation between the apical third, middle third, and coronal third of the root. Linear regression analysis was applied with the apical third as a dependent variable and middle third and coronal third as independent variables.

The association between the coronal, middle, and apical thirds of the mesial and distal roots of mandibular 1st and 2nd molars was calculated separately using the Chi-square test. Fisher's exact test was applied for the correction for expected counts <5. All the required assumptions were

fulfilled. The level of statistical significance was set at 5%. SPSS version 20 (IBM, Chicago, IL, USA) was used for all statistical analyses.

RESULTS

The descriptive statistics for mandibular 1st and 2nd molars, measuring the frequencies of all the variables, are represented in Tables 1 and 2.

In the mesial roots of mandibular 1st molars, Type IV isthmus was more frequently observed at the coronal 3rd (42.9%), whereas in the mesial roots of mandibular 2nd molars, Type V isthmus was the most prevalent at the coronal 3rd (42.9%). Type I isthmus was more prevalent in the middle 3rd (71.4%) of mesial roots of both mandibular first and second molars.

Pearson's correlation coefficient between the apical and coronal thirds resulted in an intermediate correlation of 0.525 ($P < 0.01$). An intermediate correlation coefficient of 0.469 ($P < 0.05$) was also obtained between coronal and middle thirds. A very strong correlation of 0.965 ($P < 0.01$) was determined between apical and middle thirds.

Linear regression analysis provided the following regression equation with apical third as the dependent variable. The adjusted R^2 value was estimated as 93%.

Apical third total = 0.746 middle third total

The Chi-square's test of association with Fisher's exact test of correction was performed to evaluate all pairwise combinations of the mesial and distal roots in apical, middle, and coronal thirds of mandibular 1st and 2nd molars. The results showed that these pairwise associations were statistically significant ($P < 0.05$).

DISCUSSION

The present study aimed to identify the presence and type of canal isthmuses at different levels in the mesial and distal roots of mandibular molars. An isthmus is a narrow anatomical part or passage connecting two larger structures or cavities and is considered as a lateral connection between canals of the same root. Various factors, such as different levels of dentin fusion during tooth development, the deposition of dentin over time, and the calcification, may contribute to variance in the isthmus connections, leading to the development of distinct types.[17] It is essential to

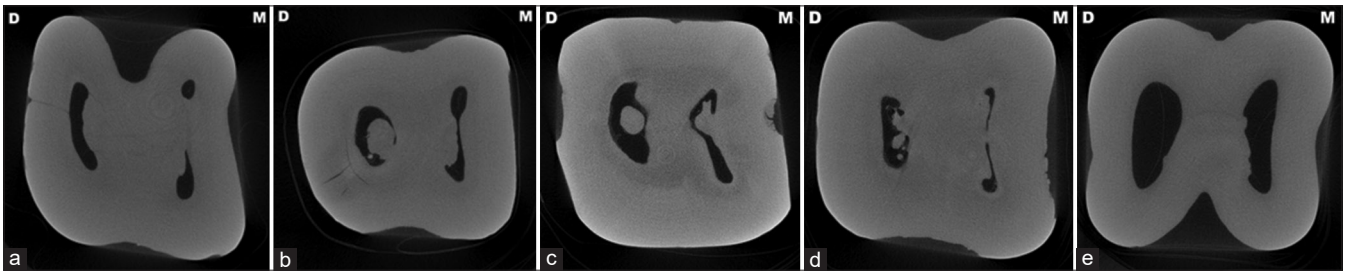

Figure 1: Types of isthmus configurations according to Hsu and Kim classification. Mesial root (M); (a) Type I, (b) Type II, (c) Type III, (d) Type IV, (e) Type V. Distal root (D); (a-e) Type V. M: Mesial root, D: Distal root

Table 1: Frequency distribution and (%) of isthmus in mandibular first molars

Type of isthmus	Groups Root	Group 1: Coronal third		Group 2: Middle third		Group 3: Apical third	
		Mesial	Distal	Mesial	Distal	Mesial	Distal
I		3 (4.8)	-	45 (71.4)	3 (4.8)	39 (61.9)	3 (4.8)
II		12 (19)	-	-	-	6 (9.5)	-
III		6 (9.5)	-	-	-	-	-
IV		27 (42.9)	9 (14.3)	15 (23.8)	6 (9.5)	12 (19)	6 (9.5)
V		9 (14.3)	30 (47.6)	3 (4.8)	36 (57.1)	6 (9.5)	33 (52.4)
VI		6 (9.5)	6 (9.5)	-	-	-	-

Table 2: Frequency distribution and (%) of isthmus in mandibular second molars

Type of isthmus	Groups Root	Group 1:Coronal third		Group 2:Middle third		Group 3:Apical third	
		Mesial	Distal	Mesial	Distal	Mesial	Distal
I		9 (21.4)	3 (7.1)	30 (71.4)	3 (7.1)	18 (42.9)	3 (7.1)
II		3 (7.1)	-	3 (7.1)	-	9 (21.4)	-
III		3 (7.1)	-	-	-	-	-
IV		3 (7.1)	-	6 (14.3)	3 (7.1)	6 (14.3)	3 (7.1)
V		18 (42.9)	21 (50)	3 (7.1)	18 (42.9)	9 (21.4)	15 (35.7)
VI		6 (14.3)	3 (7.1)	-	-	-	-

recognize that these isthmus communications are not anomalous but rather a typical feature of dental anatomy. Therefore, effective treatment must involve accessing, debriding, and disinfecting this intricate anatomy to achieve optimal treatment outcomes.

The mandibular molars are the most frequently endodontically treated teeth and present significant challenges for treatment due to complex anatomy and morphologic variations in the root canal system.[13] In recent years, μCT has emerged as an important tool for analyzing root canal morphology, providing greater resolution with 3D imaging that enables accurate visualization and analysis of intricate structures.[14,18] Therefore, this study employed μCT to investigate the prevalence and patterns of isthmi in both the mesial and distal roots of mandibular molars.

Over the years, numerous studies have attempted to investigate the prevalence of isthmus in mandibular molars. However, most of these studies have been limited to mesial roots,[7,14,15,19-21] with only one study analyzing the distal roots.[22] The buccolingually broad mesial root of the mandibular 1st molar creates an optimal environment for the formation of intercanal connections and isthmuses.[5] Most previous researches were done at the apical part of the mesial root of mandibular molars.[18] However, none of the studies reported the prevalence of isthmus in the coronal and middle third levels of mesial and distal roots, particularly in the Indian population. Therefore, to address this gap of knowledge, this is the first study of its kind that aims to evaluate isthmi prevalence and configuration in coronal, middle, and apical regions of both mesial and distal roots of mandibular molars in the Indian population.

Previous studies have reported the presence of isthmuses in the mesial root of mandibular molars, with varying prevalence rates and distribution along the root length. The prevalence rate of 73%–100% has been reported with higher incidence of isthmus observed in apical region.[19] Studies evaluating serial sections at different levels from the apex have found the highest incidence of canal isthmuses at 4–5 mm, with incidences ranging from 19% to 100%.[5,20] Any root with a compressed form or multiple canals has the potential to contain an isthmus. The canal isthmus is clinically significant in both nonsurgical root canal treatment and periapical surgery.

According to the classification of Hsu and Kim, the Type I isthmus represents no isthmus or connections, and this can be biomechanically prepared as two or three separate canals. The present study found that Type I isthmus was most frequently observed in middle and apical third of mesial roots of mandibular molars [Figure 1a]. In the Indian population, a CBCT study reported Type I isthmus was present in 38.67% of the mesial root and 12.33% of the distal root of mandibular 1st molars.[23] Another similar study on the same population reported isthmus prevalence rates of 30.34% in coronal, 15% in middle, and 4% in apical third of the mesial root of mandibular molars, respectively, without classifying the isthmus types.[24] A study using clearing technique reported Type I ranging from 3% to 18% in mesial and distal roots of mandibular molars,[8] while another μCT study showed a prevalence rate of 52.9% in the mesial root of mandibular molars.[7] The present study reported a highest prevalence rate of Type I isthmus in the middle third of the root, i.e., 71.4%, which is the highest among all the studies reported to date. The single canal was seen in distal roots of both molars ranging 18%–21%. These variations could be attributed to the factors such as ethinicity,[4,19] type of study, method of evaluation,[3,8] tooth type, and level of the root.

Type II isthmus is a definite communication between two canals, which may occasionally have dentin deposition, leading to sealing of that area. The present study found Type II isthmus exclusively in the mesial root, with a maximum occurrence of 19% in coronal third of the mandibular 1st molars, and 21.4% in the apical third of mandibular 2nd molar [Figure 1b]. Other μCT studies on different populations have reported the prevalence of Type II isthmus ranging from 7.7% to 13%.[7,8] However, one study reported a notably higher incidence of Type II isthmus in mandibular 1st molars, with a frequency of 56.33% in the mesial root and 16% in the distal root of mandibular first molars.[23]

Type III isthmus is proper or definite communication between three canals. In the current study, it was the least common type to be seen in mesial roots of 1st (9.5%) and 2nd molars (7.1%) and was not observed in distal roots [Figure 1c]. This is in accordance with a previous study by Karunakaran *et al*., who reported a 3% incidence of Type III isthmus in the Indian population.[23] Similarly, another study that used a clearing approach did not find Type III isthmus in the mesial root of the mandibular 1st molars.[8]

Type IV isthmus is partial communication but not through the entire width of the root canal, whereas Type V isthmus is complete communication running continuously between canals. Due to their unique morphological appearance, both types require special attention during biomechanical preparation, as they are known to harbor bacteria and biofilms, organic and inorganic tissues, and pulpal remnants. The treatment outcome is jeopardized, if these areas are not adequately debrided.[4] The use of ultrasonic tips will be very useful in widening these intercommunications in the coronal areas, thereby improving accessibility in the middle and apical thirds of the root. In contrast to previous study which reported 2% of Type IV isthmus in mesial root and complete absence of Type V isthmus in both mesial and distal roots of molars in the Indian population,[23] the present

study revealed a higher prevalence. The coronal third of the mesial root of the mandibular 1st molars showed the highest prevalence of Type IV (42.9%) [Figure 1d] followed by Type V isthmus (14.3%) [Figure 1e]. Furthermore, Type V was the most common isthmus in the distal root of first and second molars in the range of 40%–50%. Other studies have reported the prevalence of Type IV in the range of 15%–19.2%, and Type V in the range of 15%–17.3%.[8]

The cleaning and disinfecting of root canal interconnections and isthmuses have been identified as one of the most challenging therapeutic difficulties. These communications are often too narrow to be mechanically cleaned. Previous studies have found that between 10% and 50% of the root canal surface area remains uninstrumented. Furthermore, despite two sessions of endodontic therapy, studies have shown that the apical isthmus could not be completely free of bacteria.[25]

Numerous *in vitro* obturation studies have demonstrated that complete obturation of root canal isthmuses is unattainable. Dentinal debris generated during canal instrumentation is known to penetrate the isthmuses, which cannot be removed even with continuous irrigation during and after instrumentation. This AHTD diminishes the efficacy of irrigants and hinders the penetration of sealers and filling materials into isthmuses, thereby preventing complete sealing.[26] Therefore, to achieve successful treatment outcomes, it is recommended to employ small size files with buccolingual movement in the isthmus area, along with antibacterial irrigants with ultrasonic activation, and interappointment medicaments.[3]

In surgical endodontics, the isthmus poses a significant challenge. Contemporary endodontic surgery suggests that the optimal level for root-end excision is between 3 and 4 mm. One study reported that incomplete isthmuses were more prevalent in 6 mm apical roots.[27] In addition, a systematic review has reported that internal communications are mostly seen in the middle segments of the root, about 4–6 mm away from the apex, and has reported isthmuses in 79.5% of mesial roots and 57.5% of distal roots.[28] It is important to note that there is a high possibility that an isthmus may extend longitudinally up to middle third of the root. This has also been confirmed by our study, which found Type IV and Type V isthmuses in 23.8% and 57.1% of the mesial and distal roots of the mandibular first molars, respectively. Therefore, it is advised to perform retro-preparation and retro-filling to a depth of 3 mm after a 3 mm root end excision during periapical surgery of mandibular molars. Clinicians should be aware of the high prevalence of an isthmus and should meticulously examine resected root surfaces using high magnification with the aid of dyes.[19]

Similar to the study by Mannocci *et al*.,[29] the present μCT study also revealed nonclassifiable variations in the isthmi configurations that did not confirm to the classification of Hsu and Kim. This atypical variant exhibited a prominent buccal isthmus in addition to the isthmuses present on mesial and distal aspects. Moreover, communications were also seen projecting towards the furcal area from the middle of mesial and distal isthmuses [Figure 2a]. In another atypical variant, buccal and lingual isthmuses were also present, and they were communicating with each other in the furcal zone [Figure 2b].

The atypical variant was observed only in the coronal third, with a frequency of 7.1%–8.7% and these atypical types were not observed in the apical and middle thirds of root of mandibular molars. The Hsu and Kim classification has been criticised for considering the absence of an isthmus as Type I.[4] Furthermore, all the existing isthmus classifications do not include atypical variants, which have been found to be prevalent in certain populations,[29] such as the Indian population in our study. Thus, further research is necessary to identify and classify these atypical variants, in order to improve the accuracy and completeness of isthmus classifications.

Peiris *et al*. reported a higher prevalence of isthmus in intermediate age group and less common in both younger and older groups.[30] Conversely, Hu *et al*. found that the prevalence of isthmi declines with age and a positive correlation was observed between younger age and isthmi in the apical third region.[20] However, it should be noted that age was not considered a factor in our study, which could be considered a limitation.

A didactic method of educating dental professionals is to demonstrate them the prevalence of anatomical variances and classify them to improve clinical practise based on sound principles. Despite its limitations, this study provides valuable insights into the different types of isthmuses present at different levels of root that are encountered during routine clinic procedures. It is essential for clinicians to have a thorough understanding of the

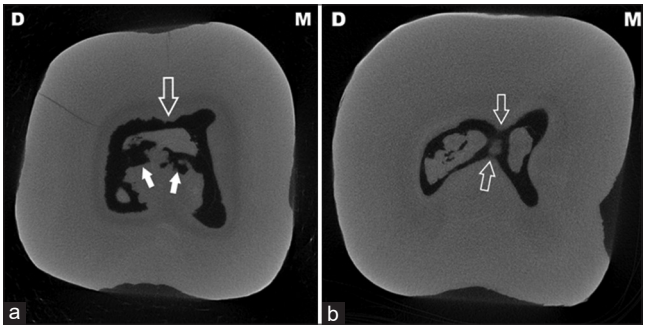

Figure 2: Atypical isthmus configurations. (a) Buccal isthmus (hollow arrow), interconnections reaching furcal area (solid arrows). (b) Buccal and lingual isthmuses (hollow arrows). M: Mesial root, D: Distal root

intricacy of the root canal system, especially the presence of isthmus, in order to provide appropriate treatment and achieve successful outcome. Future research on isthmus prevalence should consider age as factor.

CONCLUSION

This study found that the prevalence of isthmus in mesial and distal roots of mandibular 1st and 2nd molars varied by the root level and type. The presence of atypical isthmus variants not accounted for by existing isthmus classifications was also identified. The findings of this study emphasize the need for further research to identify and classify atypical isthmus variants, including the consideration of age as a factor. Overall, a thorough understanding of the root canal system and its distinct variations are crucial for providing appropriate treatment and achieving successful outcomes in endodontic therapy.

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Conflicts of interest
There are no conflicts of interest.

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