

## Original Research Article

# Types of Isthmus and Prevalence in Mandibular Incisors

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## ABSTRACT

**Aim:** This study aimed to evaluate the types of isthmus and their prevalence in mandibular incisors using magnification systems and histochemical methods.

**Materials and Methods:** The material was 150 randomly selected human mandibular incisors devoid of caries and free of any pathology in the crown or root portion. Samples were preserved in 10% formalin until needed. After fixation and decalcification, serial sections were placed into tissue-tracking cassettes, stained with hematoxylin and eosin, and examined under a light microscope at 40 $\times$  magnification.

**Results:** Of the 150 mandibular incisors examined, various types of isthmus were observed in 105. The most frequently observed isthmus type was type 1, accounting for 44.8% of cases, while the least observed was type 3, accounting for 8.6%. No isthmus was observed in 30% of the samples. Isthmuses were significantly more common at the apical 5 and 6 mm levels; however, no significant difference was observed in the types of isthmus.

**Conclusion:** Mandibular incisors exhibit significant variations in isthmuses, indicating a complex structure. Knowledge of the possible location and variations of isthmuses is crucial as it not only influences the selection of techniques and instruments but also directly impacts treatment success. Standard instrumentation techniques alone may not suffice for thoroughly cleaning the root canal system in complex cases, such as those with isthmus variation.

**Keywords:** Histological section; Isthmus; Mandibular incisor

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## INTRODUCTION

Success in endodontic therapy is predicated upon the thorough debridement, shaping, and obturation of the root canal system. Effective endodontic treatment necessitates not only a profound understanding of the primary morphology of the root canal but also an awareness of potential anatomical variations within the system. These variations encompass lateral canals, accessory canals, C-shaped canals, apical ramifications at the root apex, and isthmus.<sup>1</sup>

Isthmuses are of particular interest due to their prevalence, their anatomical variations, and the challenges they pose during root canal treatment procedures, which can lead to clinical difficulties. These complex structures within the root canal anatomy can serve as reservoirs for remnants of necrotic pulp tissue and bacteria. Inadequate mechanical debridement of these areas can result in unsuccessful endodontic treatment outcomes.<sup>2</sup>

Cambruzzi and Marshall<sup>3</sup> first introduced the concept of the isthmus in endodontics in a 1978 article published in the Canadian Dental Journal. Detailed studies of isthmus-related endodontic treatment began appearing in the literature around 1990. An isthmus is defined as a narrow, ribbon-like connection between two root canals within the same tooth. The isthmus structure, which contains pulp or pulp-associated tissues, forms when Hertwig's epithelial root sheath fails to close completely during tooth development in the embryonic period.

An isthmus connection can be observed between any two root canal systems within a single root. Partial fusion of root projections results in the formation of

two root canals interconnected by an isthmus, such as in the mesial root of the mandibular first molar. Without fusion, a large ribbon-shaped canal may form, creating an isthmus that extends throughout the entire root.<sup>4</sup>

In conclusion, complete fusion of adjacent root projections results in the formation of a single root with a unified root canal system, as exemplified by the distobuccal roots of maxillary molars. In contrast, partial fusion can lead to the formation of two root canals interconnected by an isthmus, as observed in the mesial roots of mandibular first molars.

Many different classifications have been proposed for isthmus. One of the most commonly used is the classification proposed by Hsu and Kim in 1997<sup>4</sup>, which consists of five types of isthmus. This study used this classification to define isthmus as follows: Type I, an incomplete isthmus characterized by a faint communication between two canals; Type II, a definite connection between two canals, constituting a complete isthmus; Type III, a very short complete isthmus between two canals; Type IV, a complete or incomplete isthmus among three or more canals; and Type V, two or three canal openings without a visible connection.<sup>5,6</sup>

In mandibular incisors, endodontic treatment failure has been associated with an untreated lingual canal or an untreated isthmus.<sup>7</sup> The frequency of two canals in mandibular incisors has been reported to range from 11.5% to 44.1%. However, in most roots, these canals merge into a single canal within the apical 1–3 mm.<sup>8,9</sup>

Periapical surgical procedures, such as root-end resection, can reveal the presence of a second canal or isthmus. Surgical microscopy and ultrasonic root-end preparation provide clinicians with enhanced visualization during these procedures. Knowledge of potential variations in root canal anatomy is crucial for increasing the likelihood of successful treatment.<sup>8</sup>

Literature reviews have shown that studies on variations in isthmus and canal morphology have been conducted predominantly on molar teeth.<sup>5</sup> Limited information is available on the anatomical configuration of the pulp in mandibular incisors, and opinions differ among existing studies.<sup>10</sup>

This study aimed to contribute to the literature by evaluating the types of isthmus and their prevalence in the apical 8 mm of mandibular incisors. These isthmuses are commonly encountered during endodontic treatment and apical surgery, and their presence can significantly affect the prognosis of these procedures.

## MATERIALS AND METHODS

This study was approved by the Ethics Committee of Çankırı Karatekin University (February 5, 2024, Meeting No: 11).

### Selection of Sample Teeth

One hundred and fifty human mandibular incisors, extracted within the last six months for various reasons and devoid of caries, were randomly collected and preserved in 10% formalin. No distinction was made between mandibular lateral and central incisors. Patients' age, gender, and ethnicity were not recorded. Buccolingual and mesiodistal radiographs (CSN Industry, Italy) were obtained for all teeth. Teeth exhibiting incomplete root development, caries, cracks, fractures, compromised root integrity, pathologies such as calcification or root resorption, or prior root canal treatments were excluded from this study.

### Preparation of Sample Teeth

Soft tissue remnants were meticulously removed from the sample teeth using a lancet. Calculus and hard tissue debris were removed with a scaler. The crowns were sectioned at the cementoenamel junction using a flame-shaped carbide bur mounted on an air turbine handpiece with continuous water cooling. In order to ensure proper preservation of the pulp tissue, the teeth were stored in a 10% formalin solution until needed.

### Histological Evaluation

After fixation, the samples were placed in containers with 10% formic acid for decalcification and kept at room temperature for four weeks. After decalcification, the samples were sectioned into 1 mm slices starting from the apex using a microtome (Leica Biosystems, Germany), resulting in eight sections per sample. Two researchers examined these sections using a dental loupe (ExamVision ApS, Denmark) at 2.8×

magnification. The localization and types of isthmus observed were documented.

During the examination with the dental loupe, suspected fractures were identified in samples from three teeth. It was hypothesized that these might have resulted from the decalcification and fixation processes. To definitively distinguish between potential fracture lines and isthmus structures, the samples were further examined using scanning electron microscopy (SEM; Sigma 300vp; Zeiss).

The sections examined with the dental loupe were subsequently placed in tissue cassettes for histological processing, which was conducted over 12 hours using a tissue processing device. Sections (3–5  $\mu\text{m}$  thick) were sliced from each paraffin-embedded sample using a microtome, stained with hematoxylin and eosin, and examined under a light microscope (BX60; Olympus, Japan) at 40 $\times$  magnification. Two researchers examined the resulting images. The isthmus types in the samples were identified according to Hsu and Kim's classification.

### Statistical Analysis

Statistical analyses were performed using the R statistical software in the RStudio environment (version 2023.06.0+421).<sup>11,12</sup> The impact of observed isthmus type and root section level factors on the frequency of isthmus occurrence was assessed using the Fisher's exact test. Differences in the occurrence of isthmus types were assessed using

the Chi-Square Goodness-of-Fit test to determine whether some types were observed significantly more frequently than others. A p-value of  $<0.05$  was considered statistically significant.

## RESULTS

Among the 150 mandibular incisors examined, isthmus variations were identified in 42, encompassing 105 sections. A type 1 isthmus was observed in 44.8% of the sections ( $n = 47$ ), type 5 in 24.8% ( $n = 26$ ), type 2 in 21.9% ( $n = 23$ ), and type 3 in 8.6% ( $n = 9$ ). No isthmus variation was observed in 72% of the examined samples ( $n = 108$ ).

No isthmus variation was observed in the apical 1–2 mm of the samples. In five samples, no canal was observed in the apical 1–2 mm sections. While isthmuses were most commonly observed in the mid-thirds of the teeth (5–6 mm), isthmus counts did not differ significantly among different section levels ( $p = 0.841$ , Fisher's Exact test).

The most frequent isthmus type was type 1, while the least frequent was type 3. Counts differed significantly among the different isthmus types (Chi-Square Goodness-of-Fit test,  $p < 0.001$ ) (Figure 1). Additionally, different types of isthmus variations were detected in different sections of the same tooth in nine samples (Figures 2 and 3). In the samples subjected to SEM analysis, a fracture line was detected in the tooth alongside type 1, type 2, and type 4 isthmuses (Figures 4–6).

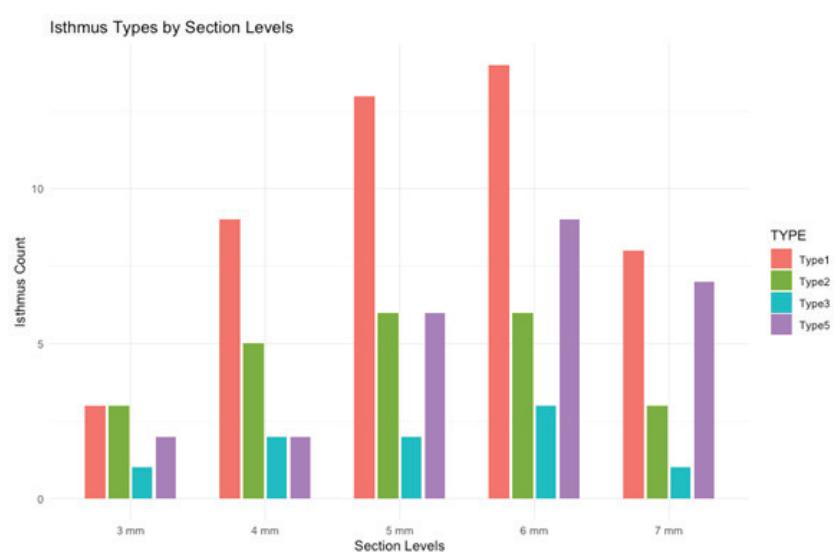
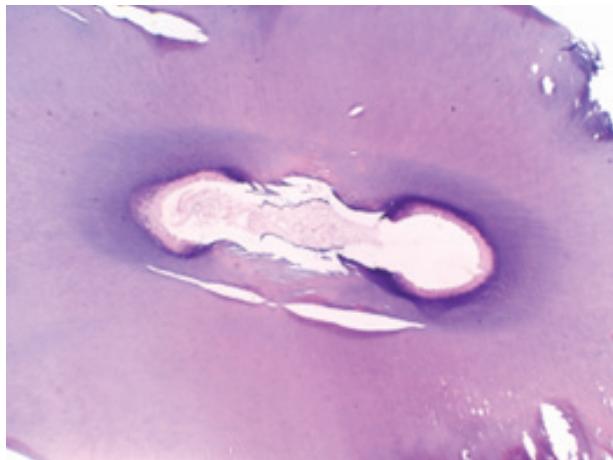
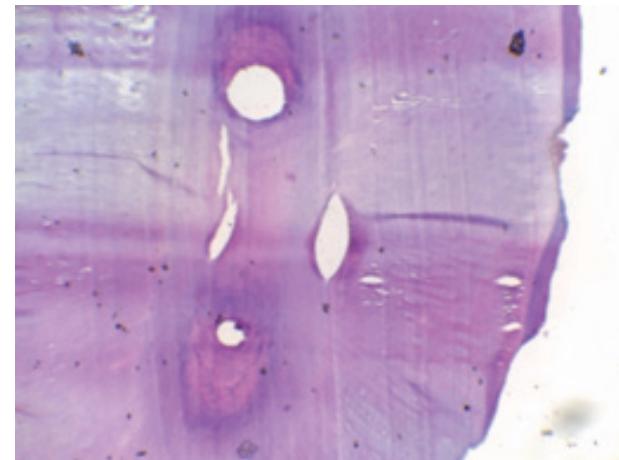


Figure 1. Isthmus types by section level.



**Figure 2.** Light microscopy image of a Type 3 isthmus.



**Figure 3.** Light microscopy image of a Type 5 isthmus.



**Figure 4.** Scanning electron microscopy image of a Type 1 isthmus.



**Figure 5.** Scanning electron microscopy image of a Type 2 isthmus.



**Figure 6.** Scanning electron microscopy image of a Type 4 isthmus.

## DISCUSSION

Understanding the normal configuration of the pulp space and the variations in canal anatomy is vital to the success of endodontic treatment. Isthmus, anatomical variations of clinical significance play a critical role in non-surgical endodontic treatment and periradicular surgery.<sup>6</sup> The presence of an isthmus may adversely affect the outcome of root canal treatment, as these narrow and anatomically complex areas often serve as reservoirs for residual pulp tissue and microorganisms. Conventional instrumentation techniques are insufficient to thoroughly access and debride these regions, thereby necessitating the implementation of advanced irrigation protocols to achieve effective disinfection.<sup>1,13</sup> Failures in non-surgical root canal treatment are often attributed to inadequate debridement of these areas. In some cases, periapical surgery is needed to facilitate the cleaning and sealing of the root apex.

A retrospective study evaluating the outcomes of periapical surgical treatment revealed a significantly higher success rate in maxillary teeth compared to mandibular teeth.<sup>14</sup> This discrepancy may be associated with the higher prevalence of isthmus variations in mandibular anterior teeth compared to maxillary teeth. However, other factors, such as differences in bone density, root-end filling materials, and tooth location, may also contribute. Successful apical surgery outcomes hinge on the accurate diagnosis of isthmus using magnification systems, appropriate ultrasonic applications, and retrograde filling with leak-proof, biocompatible materials.<sup>15</sup> Furthermore, isthmus areas create a major challenge in achieving a void-free obturation. Due to their narrow and irregular shape, these areas frequently resist complete sealing, thereby acting as potential pathways for leakage or reinfection.<sup>3</sup>

One study reported that the probability of observing two canals in mandibular incisors ranges from 11.5% to 44.1%, with most roots merging into a single canal in the apical 1–3 mm.<sup>16</sup> The absence of isthmus in the apical 2 mm in our study supports this finding. Additionally, no canal was observed in the apical 2 mm section in five dental samples evaluated in our study, likely due to the apical foramen not always opening at the anatomical apex.<sup>17</sup> Given the lack of significant differences between genders and age

groups in other studies, our study did not attempt to stratify the results by gender, race, or age.<sup>18</sup>

Literature reviews indicate that studies evaluating isthmus prevalence and types have often focused on molar teeth.<sup>19–21</sup> Few studies have examined isthmus structures in mandibular incisors. However, undetected lingual canals or untreated isthmus are recognized as significant contributors to endodontic failure in these teeth.<sup>7</sup>

Mauger *et al.*<sup>8</sup> found isthmus structures in 22% of examined mandibular incisors, compared to 28% in our study. The discrepancy might be due to differences in the evaluated apical length, with Mauger *et al.*<sup>8</sup> examining the apical 3 mm compared to our study's evaluation of the apical 8 mm. Additionally, the smaller sample size in Mauger *et al.* (n = 100) compared to our study (n = 150) may have influenced the results.

Another study reported relatively high success rates (>85%) for anterior maxillary and mandibular teeth, while mandibular molar teeth exhibited a lower success rate of 63.7%.<sup>22</sup> This lower success rate can be attributed to the complex endodontic anatomy and difficulty in accessing the apices of mandibular molars. Apical resection procedures are often performed in studies involving anterior teeth due to easier access and visibility, particularly in the maxillary region.<sup>8</sup> Therefore, further studies explicitly evaluating the prognosis of mandibular anterior teeth are warranted.

In *in vitro* studies examining isthmus prevalence, the isthmus can be damaged during tooth sectioning. The smear layer generated during sectioning can obstruct the isthmus, leading to inaccurate evaluations. Our study encountered similar challenges, which were mitigated by examining some samples using SEM.

Our study demonstrated that mandibular incisors exhibit various isthmus configurations, indicating a complex anatomical structure. Knowledge of potential variations encountered in the root canal is crucial, as it influences the selection of techniques and instruments during treatment, directly impacting treatment success. Given the intricate nature of isthmus-containing systems, conventional shaping and filling techniques may not sufficiently address these spaces, highlighting the necessity

of magnification, targeted ultrasonic instruments and optimized irrigation protocols.<sup>1,3</sup> Standard instrumentation techniques alone are insufficient for cleaning the root canal system in cases with variations such as isthmus. The identified isthmus should be included in the root-end preparation during periapical surgical interventions. Advanced magnification and illumination systems, such as surgical operating microscopes, are essential for locating additional canals and identifying isthmuses. Furthermore, the potential impact of isthmus variations must be considered when investigating the etiology of treatment failures.

The limitations of our study include its use of magnification systems, digital radiography, histological evaluation, and SEM methods. Its efficacy could have been enhanced by incorporating three-dimensional imaging systems. The anatomical evaluation data could also be further supported by broader time intervals and more extensive archival scans.

## CONCLUSION

Our study demonstrated that mandibular incisors exhibit various isthmus configurations, indicating a complex anatomical structure. Knowledge of potential variations encountered in the root canal is crucial, as it influences the selection of techniques and instruments during treatment, directly impacting treatment success. Standard instrumentation techniques alone are insufficient for cleaning the root canal system in cases with variations such as isthmuses. The identified isthmus should be included in the root-end preparation during periapical surgical interventions. Advanced magnification and illumination systems, such as surgical operating microscopes, are essential for locating additional root canals and identifying isthmuses. Furthermore, the potential impact of isthmus modifications must be considered when investigating the etiology of treatment failures.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## FINANCIAL DISCLOSURE

The authors declared that they received no financial support.

# Mandibular İnsizörlerde Görülen İsthmus Tipleri ve Prevalansı

## ÖZET

**Amaç:** Bu çalışmanın amacı, mandibular keser dişlerin sahip olduğu isthmus tiplerinin ve sıklığının büyütme sistemi ve histokimyasal yöntemler yardımıyla değerlendirilmesidir.

**Gereç ve Yöntem:** 150 adet rastgele seçilmiş insan mandibular keser dişi kullanılmıştır. Kök ucu gelişimi tamamlanmış, kırık, çürük, çatlak olmayan, kanal tedavisi girişiminde bulunulmamış, kanal içerisinde herhangi bir patoloji varlığı tespit edilmemiş dişler çalışmaya dahil edilmiştir. Kuronları uzaklaştırılmış olan kökler, kullanım zamanına kadar %10 formalin içerisinde bekletilmiştir. Fiksasyon ve dekalsifikasyon işlemlerinin ardından, sıralı kesitleri doku takip kasetleri içerisinde alınan örnekler Hematoksilen-Eozin ile boyanmış; ardından ışık mikroskopunda x40 büyütme altında incelenmiştir.

**Bulgular:** İncelenen 150 adet mandibular keser dişin 105'inde çeşitli isthmus tiplerine rastlandı. En fazla görülen isthmus tipi %44,8 oranında Tip 1; en az %8,6 oranında Tip 3 isthmus görüldü. Örneklerin %30'unda herhangi bir isthmusa rastlanmadı. İsthmusların en fazla görüldüğü apikal 5mm ve 6 mm kesitleri istatistiksel olarak anlamlı bulundu; görülen isthmus tipleri bakımından ise bir anlamlılık tespit edilmedi.

**Sonuç:** Mandibular keser dişler istatistiksel olarak anlamlı düzeyde isthmus varyasyonuna sahip, kompleks yapı göstermektedir. İsthmusun kök kanalında hangi lokasyonda olabileceği ve hangi varyasyona sahip olacağı ile ilgili bilgi sahibi olunması; yapılacak tedavi sırasında kullanılacak olan teknik ve enstrüman seçimini etkileyeceğ gibi tedavinin başarısı üzerinde de direkt rol oynayacaktır. İsthmus varyasyonu gibi kompleksite gösteren durumlarda, standart enstrümantasyon teknikleri tek başına kök kanal sisteminin temizlenmesinde yeterli olmayacağı.

**Anahtar Kelimeler:** Histolojik kesit; İsthmus; Mandibular keser

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