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Prevalence of MB2 canals in maxillary molars using different assessment methods: ex vivo analysis

Prevalência de canais MB2 em molares superiores usando diferentes métodos de avaliação: análise ex vivo

Prevalencia de canales MB2 en molares maxilares utilizando diferentes métodos de evaluación: análisis ex vivo

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Abstract
The anatomical complexity of the root canal system of the maxillary molars is considered a challenge to endodontic treatment. The aim of this study was to compare different diagnostic methods for identification of MB2: clinical examination (CE), dental operating microscope (DOM), digital periapical radiography (DR), cone-beam computed tomography (CBCT) and cross sections (CS). Sixty-one maxillary molars were randomly selected. Initially axial images were performed using CBCT. DR were made in ortho-positions, mesial positions and distal positions. The images were evaluated by an experienced examiner, the data were tabulated and not being revealed until the end of the experiment. After those openings and conventional coronary access was made, and the teeth evaluated by CE. Then the teeth were evaluated by DOM. The variable studied presents nominal and dichotomous nature ("absence of MB2 canal" and "presence of MB2 canal"). The agreement between the methods, when compared by pairs, was calculated by Cohen's Kappa. A major percentual of MB2 detection was obtained by CBCT (67%), follow by CS (55%) and DOM (45%). The concordance between CS and CBCT was substantial (Kappa=0.76; 95%CI: 0.59 to 0.92); between CBCT and DOM was fair (Kappa=0.32; 95%CI: 0.09 to 0.56), as well as between DOM and CE. All the other concordance analysis showed slight agreement (Kappa from 0.00 to 0.20). The identification of MB2 can be facilitated using CBCT and DOM.
Keywords: Anatomy; Cone-beam computed tomography; Endodontics; Molar; Radiography.

Resumo
A complexidade anatômica do sistema de canais radiculares dos molares superiores é considerada um desafio para o tratamento endodôntico. O objetivo deste estudo foi comparar diferentes métodos diagnósticos para identificação de MB2: exame clínico (CE), microscópio cirúrgico odontológico (DOM), radiografia periapical digital (DR), tomografia computadorizada de feixe cônico (TCFC) e cortes transversais (CS). Para esse estudo, sessenta e um molares superiores foram selecionados aleatoriamente. Inicialmente as imagens axiais foram realizadas usando

CBCT. As RD foram feitas nas posições orto, mesial e distal. As imagens foram avaliadas por um examinador experiente, os dados foram tabulados e não foram revelados até o final do experimento. Após essas aberturas e o acesso coronário convencional foi feito, e os dentes avaliados por CE. Em seguida, os dentes foram avaliados por DOM. A variável estudada apresenta natureza nominal e dicotômica ("ausência de canal MB2" e "presença de canal MB2"). A concordância entre os métodos, quando comparados aos pares, foi calculada pelo Kappa de Cohen. O maior percentual de detecção de MB2 foi obtido por CBCT (67%), seguido por CS (55%) e DOM (45%). A concordância entre CS e CBCT foi substancial (Kappa=0,76; IC 95%: 0,59 a 0,92); entre CBCT e DOM foi razoável (Kappa=0,32; IC 95%: 0,09 a 0,56), assim como entre DOM e CE. Todas as demais análises de concordância mostraram concordância discreta (Kappa de 0,00 a 0,20). A identificação de MB2 pode ser facilitada usando CBCT e DOM.
Palavras-chave: Anatomia; Endodontia; Tomografia computadorizada cone beam; Molar; Radiografia.

Resumen
La complejidad anatómica del sistema de conductos radiculares de los molares superiores se considera un desafío para el tratamiento endodóntico. El objetivo de este estudio fue comparar diferentes métodos de diagnóstico para la identificación de MB2: examen clínico (CE), microscopio quirúrgico dental (DOM), radiografía periapical digital (DR), tomografía computarizada de haz cónico (CBCT) y secciones transversales (CS). Sesenta y un molares maxilares fueron seleccionados al azar. Inicialmente, las imágenes axiales se realizaron mediante CBCT. Las DR se realizaron en ortoposiciones, posiciones mesiales y posiciones distales. Las imágenes fueron evaluadas por un examinador experimentado, los datos fueron tabulados y no se revelaron hasta el final del experimento. Después de esas aperturas y accesos coronarios convencionales se realizó, y los dientes evaluados por CE. Luego los dientes fueron evaluados por DOM. La variable estudiada presenta carácter nominal y dicotómico ("ausencia de canal MB2" y "presencia de canal MB2"). La concordancia entre los métodos, cuando comparados por pares, fue calculada por el Kappa de Cohen. El mayor porcentaje de detección de MB2 lo obtuvo CBCT (67%), seguido de CS (55%) y DOM (45%). La concordancia entre CS y CBCT fue sustancial (Kappa=0,76; IC95%: 0,59 a 0,92); entre CBCT y DOM fue regular (Kappa=0,32; IC95%: 0,09 a 0,56), así como entre DOM y CE. Todos los demás análisis de concordancia mostraron una ligera concordancia (Kappa de 0,00 a 0,20). La identificación de MB2 se puede facilitar utilizando CBCT y DOM.
Palabras clave: Anatomía; Endodoncia; Tomografía computarizada de haz cónico; Molar; Radiografía.

1. Introduction

The knowledge of the anatomy of root canal systems is essential to endodontic treatment successful (Machado et al., 2021; Souza Júnior et al., 2021). Complex dental anatomy represents challenge to endodontists, and one of the most likely causes of persistence or development of periradicular disease, when endodontic treatment is failure to locate, clean, shape, and obturate the root canals (Ahmad et al., 2014; Baratto-Filho et al., 2009; Pereira et al., 2021). In this context, the maxillary molars have complex internal anatomy specially because the high prevalence of three roots and four canals in these teeth. A second canal is usually found in the mesiobuccal root (MB), named MB2 (Gupta et al., 2017; Pereira et al., 2021; Wu et al., 2017).

Clinical and radiographic examination are traditional methods used to identification of root canals (Vasundhara, Lashkari, 2017; Tassoker et al., 2018). However, its efficacy depends on the knowledge and skill of the examiner (Tassoker et al., 2018), and radiography is still limited by technical factors such as contrast, density, and angulation (Zand et al., 2017). The use of visual magnification with microscope or dental loupes of the root canal can improve the ability to detect canals. Some studies have shown an increase in the success of endodontic treatment due to visual magnification (Acar et al., 2015; Vasundhara, Lashkari, 2017). Buhrley et al. (2002) compared the clinical inspection, the use of dental loupes or microscope for MB2 identification in maxillary molars and concluded that there was a significant difference in the location of the fourth canal when visual magnification was used.

Cone bean computed tomography presents a new technology, because different from clinical examination and digital radiography, allows the operator to visualize the morphological characteristics of the sample in three dimensions (Silva et al., 2022). Despite limitations such as high cost, higher radiation dose, technical knowledge for interpretation of the test, CBCT is becoming increasingly common in dental practice and is suggested as an auxiliary means for identification and diagnosis of canals and isthmus (Machado et al., 2021; Michelotto et al., 2021; Souza Júnior et al., 2021).

In context, with the technological advance several techniques appeared to facilitate the localization of the accessory canals, reducing the failure and the necessity of the retreatment of the root canals. Thus, the present study aimed compare different diagnostic methods (clinical visualization, periapical digital radiography, operative microscope, CBCT) with the real situation of the root through the cross sections for the identification of MB2 canal in the mesiobuccal root of the first and second maxillary molars.

2. Methodology

This study was approved by the Institutional Review Board of the Pontifical Catholic University of Minas Gerais (PUC Minas) (1.553.936). This is an experimental investigation in the laboratory with extracted teeth obtained from a Biobank of human teeth.

Tooth Selection and Preparation

First and second human maxillary molars were randomly collected from the Dental Department Human Tooth Bank and then exposed to digital periapical radiography in the buccolingual direction. Patient age, gender, and race were not considered. Only teeth with root formation complete and 3 separate roots were included. Teeth presenting previous endodontic treatment, accentuated radicular curvature, calcified canals or internal root resorption were excluded. After careful inspection, sixty-one maxillary molars were selected.

CBCT images

For CBCT examinations was used Kodak 9000C 3D® (Carestream Health, Inc.). The teeth were included in methylmethacrylate polymer plates (UNIGEL, São Paulo, Brazil) with silicone condensation Perfil (Coltene, Rio de Janeiro, Brazil. The exposure factors used were 60 kV, 10 mA, 10.8 seconds of exposure and scanning performed with 5.0 cm x 3.7 cm collimation (FOV) and isotropic voxel of 76 x 76 x 76 mm thick.

CBCT Image Reading Image interpretation was performed by a specialist in Dental Radiology and Imaging, with extensive experience in the interpretation of radiographic and tomographic images (Acar et al., 2015; Baratto Filho et al., 2009). For the interpretation of CT scans was used CS 3.2.9 Imaging software - Kodak Dental Imaging Software® (Carestream Health, Inc. Atlanta, USA) and allowed to use all available resources was used for image display with a display resolution 1920x1080 pixels with the brightness and contrast levels were set in their preset configuration. A voxel size of 76 μm was used with a bit depth of 15 bits. Settings were 68 kVp, 6.3 mA, and 11 seconds. Volume-renderings and multiplane volume reconstructions were performed using Carestream imaging software version (2.4.11). The CT scans were interpreted randomly and at different times. The teeth were evaluated in the axial section. The examiner considered as MB2 canal when from the pulp chamber was located hypodense one point adjacent to MB1 canal buccal-palatal sense and this was also found in the middle and apical third.

Digital radiography images

A template of Adsil Silicone (Coltene, Rio de Janeiro, Brazil) was made for each pair of teeth and standardized in accordance with the inner measurements of the methacrylate. The teeth were fixed on the plates, and the digital sensor Shick Elite® (Sirona, New York, United States) was stuck underneath. For all radiographic technique was exposed at -30°, 0° and 30° horizontal angulations and 90° vertical. The exposure factors used were 60 kV, 7mA and the exposure time was as recommended by the manufacturer (0.143 seconds). The images acquired in mesioangular, ortoangular and distoangular position were evaluated using the software CDR DICOM (Schick Technologies Inc, New York, United States Inc.).

The image interpretation was performed by a specialist in Dental Radiology and Imaging. Kodak 2200 Intraoral X-ray System (Carestream Health, Inc.) was used to acquire images with a #2-size Scan-X Duo® (AirTechniques). Each image contained 2.76 megapixels. The pixel size was 1.08 microns, the grey-level dynamic range was 12 bits, and the spatial resolution was > 20-line pairs/mm. Exposures were made at 70 kVp and 7 mA, with a nominal focal spot size of 0.6 mm, a focal distance of 4 cm, and an exposure time of 0.18 seconds. To avoid visual fatigue and impaired reading tests, the analysis was limited to 12 images in each evaluation. The radiographs were interpreted randomly and at different times. The examiner did not consider MB2 presence when was observed in 75% of its radiolucency of the pulp chamber toward close to the apex.

Clinical method

After coronal access preparation under copious irrigation with sodium hypochlorite 5.25% (Lenza Pharmaceuticals, Belo Horizonte, Brazil) the mesiobuccal canal (MB1) orifices on the pulp floor was located. Then, a sharp endodontic explorer Rhein type (Odous - ref: 100, Belo Horizonte, Brazil) was used in the vestibule-palatal direction to find the MB2 canals. When the probe explorer became attached to any part on this path this was considered as a possible canal. Thus, the sample remained intact for the next test.

Dental operating microscope (DOM)

The microscope (DF Vasconcellos, São Paulo, Brazil) was used operating with 16x of magnification, according to the same criteria of the clinical examination. Excess dentin observed in the vestibular-palatine path was removed with ultrasonic insert (ENAC - Osada, Inc, Tokyo, Japan) with tip TU17 (Triniti, São Paulo, Brazil) in low power. The location of the MB2 canal was completed with the negotiation with Kerr type files, special series (8, 10 - Maillefer, Ballaigues, Switzerland). Was only considered as MB2 canal when was fully exploited, ie when the files reached the length of patency of the independent canal or by fusion with the mesiobuccal canal, which was confirmed by radiographic examination.

Cross Sections

Finally, the specimens were evaluated by cross sections, with was considered our control method (Weine et al., 2012). To obtain the precise cross sections (CS) and homogeneous diameter, the MB roots were individualized and fixed in acrylic plates to allow them to be sectioned serially in the direction perpendicular to the long axis of the tooth with the Isomet® 1000 machine (Buehler, Lake Forest, USA), using a 0.5 mm diamond disk (South Bay Technology, San Clement, CA, USA) under refrigeration, obtaining 3 slices with 1.0 mm thickness for each third of the root (cervical, medium and apical). For the analysis of the sections, one slice of each third was selected and evaluated using the Olympus® FSX100 digital microscope (Olympus, Tokyo, Japan), with an increase of 4.2x. The examiner considered MB2 canal when the canal was present in at least two sections.

Statistical analysis

The variable studied presents nominal and dichotomous nature ("absence of MB2 canal" and "presence of MB2 canal"). The agreement between the methods, when compared by pairs, was calculated by Cohen's Kappa. Concordance analyses were achieved by StatsToDo statistical programs on the website www.statstodo.com (StatsToDo Trading Pty Ltd, Brisbane, QLD, Australia). The results were compared to the strength of agreement related to the kappa statistics proposed by Landis and Koch (1977):

- Kappa < 0.00: poor agreement.
- Kappa from 0.00 to 0.20: slight agreement.

- Kappa from 0.21 to 0.40: fair agreement.
- Kappa from 0.41 to 0.60: moderate agreement.
- Kappa from 0.61 to 0.80: substantial agreement.
- Kappa from 0.81 to 1.00: almost perfect agreement.

3. Results and Discussion

The results of the presence of MB2 canal detected in each method can be observed in the Table 1.

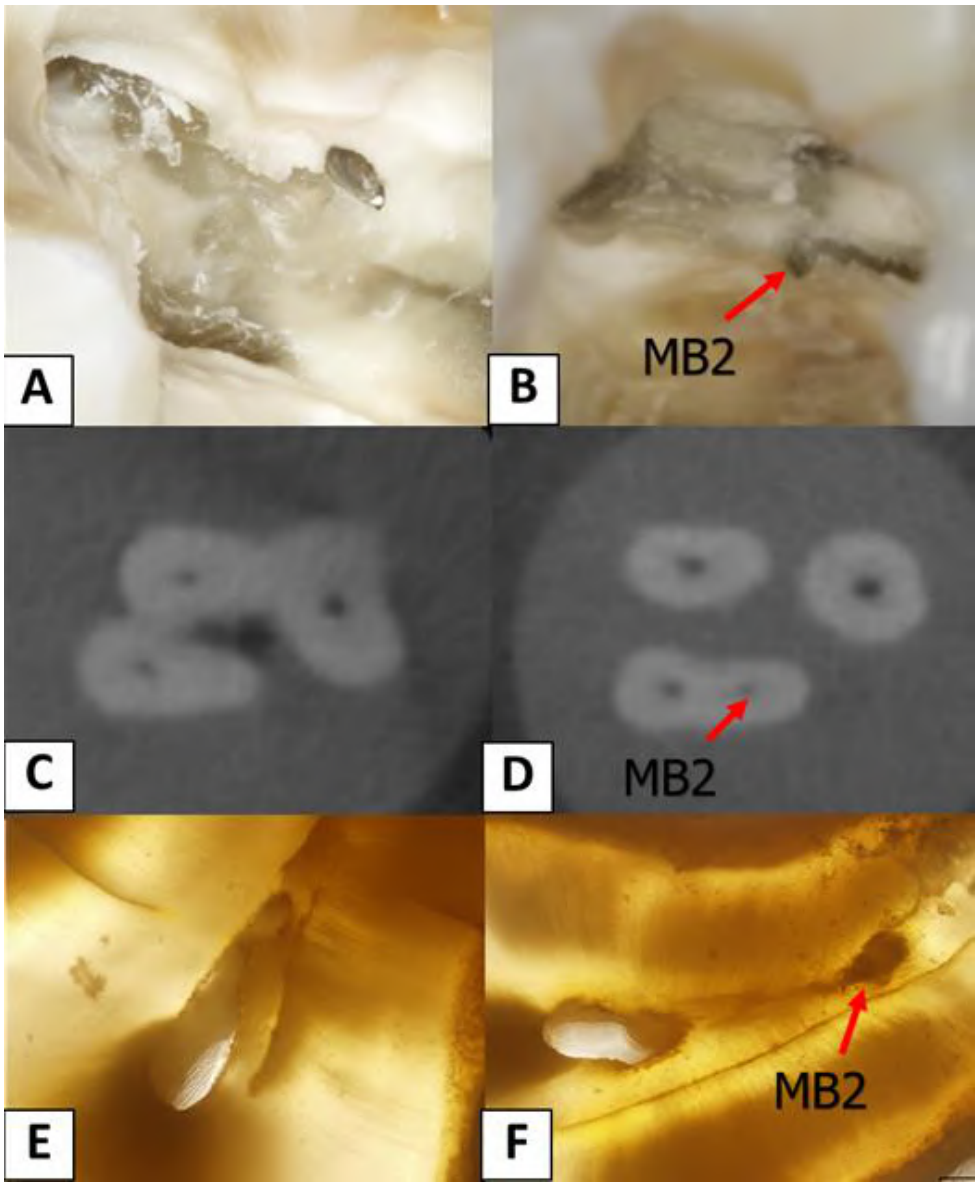
Table 1. Data from MB2 canal detect in maxillary molars for each method.

Method	**MB2 detect**	**%**
CM	13	21
DOM	28	45
DR	8	13
CBCT	41	67
CS (control method)	34	55

Source: Authors.

The concordance between CS (control method) and CBCT was substantial (Kappa=0.76; 95%CI: 0.59 to 0.92). The concordance was fair between CBCT and DOM (Kappa=0.32; 95%CI: 0.09 to 0.56) as well as between DOM and Clinical Method (Kappa=0.27; 95%CI: 0.02 to 0.52). All the other concordance analysis between the methods showed slight agreement (Kappa from 0.00 to 0.20). The Figure 1 shows images highlighting the absence or presence of MB2 canal, by using some method of assessment of this study.

Figure. 1. A e B – Dental Operating Microscopy images; C e D- CBCT images; E e F – Cross sections images, showing absence and presence of MB2, respectively.



Source: Authors.

In comparison, Figures 1 A, C and E demonstrate shows maxillary molars with the absence of MB2 canal and in B, D and F, with its presence in the clinical, tomographic and sectioning images evaluated with magnification methods, respectively.

The variation in the morphology of the root canal system in multiradicular teeth remains challenge in Endodontics (Machado et al., 2021; Ordinola-Zapata et al., 2017; Pereira et al., 2021). With technological advancement the CBCT, DR and visual magnification are becoming a reality in successful endodontic treatment, as they allow considerable advantages in terms of quality, efficiency, and security to clinical and specialist. For this reason, studies correlating these issues are of major importance in endodontic practice (Ahmad et al., 2014; Ordinola-Zapata et al., 2017; Silva et al., 2022).

Historically, the maxillary molars have been widely studied because of the complexity of their internal anatomy (Baratto Filho et al., 2009; Seidberg et al., 1973; Weine et al., 2012). Particular attention has been given to the MB root, whose flattening in the mesiolingual direction provides a high prevalence of two canals. In the literature, the frequency of MB2 in

maxillary molars ranges from 25% to 100% (Ahmed et al., 2017; Buhrley et al., 2002; Gupta et al., 2017; Pereira et al., 2021; Wolf et al., 2017).

Differences between studies can be explained by the variation in sample size, ethnicity, and diversification of methods and techniques employed (Baratto Filho et al., 2009; Studebaker et al., 2017). Other factors, such as age and gender, also seem to interfere with these results (Abella et al., 2015; Ahmed et al., 2017; Olczak et al., 2017; Ratanajirasut et al., 2018). In addition, the present study did not consider the differences between the first and second maxillary molars, while the last may imply a lower frequency of MB2 (Acar et al., 2015; Buhrley et al., 2002; Olczak et al., 2017; Wolf et al., 2017).

Although MB2 canal identification is technically difficult during endodontic treatment with only human eye, it is still performed (Acar et al., 2015; Vasundhara, Lashkari, 2017). Although DOM has identified fewer MB2 compared to CBCT in the present study, 45% e 67%, respectively, both showed fair concordance (0.32 - 95%CI: 0.09 to 0.56). In a clinical study, Buhrley et al. (2002) investigated the effect of magnification on the incidence of MB2 assessment. The MB2 canal was found in 41 of 58 teeth (71.1%) when using SOM. When using loupes MB2 was identified in 55 of 88 teeth (62.5%). The lowest incidence of MB2 was in the group without any magnification, when MB2 was found in only 10 of 58 teeth (17.2%). These results were like the present study, supporting importance of magnificence in endodontic practice.

Other studies have shown the same increase in the prevalence of this canal with the use of lenses, magnifiers, and microscopes (Ratanajirasut et al., 2018; Tassoker et al., 2018). In addition, adequate coronary access when combined the DOM to the ultrasonic tips, especially in the pulp floor chamber and in the isthmus regions promotes a significant increase of MB2 canal detection (Acar et al., 2015; Buhrley et al., 2002).

In the present study, the occurrence of MB2 detected by digital radiographic was low (13%). It can be explained because the mesiobuccal root of the maxillary molar is relatively slender mesiodistally and broad buccolingually. It represents a difficult to visualize canal anatomy that have complex 3D anatomy and representations of this anatomy provided by 2D intraoral radiographs contain little information on the buccolingual dimension (Buhrluey et al., 2002; Zand et al., 2017). Canals that are aligned in a buccolingual plane cannot be easily differentiated from each other. In addition, superimposition of anatomical structures and image distortion, especially in the maxilla, often obscure canal anatomy (Torres et al., 2015; Zand et al., 2017). Zand et al. (2017) also evidenced a significant statistical difference between the results of periapical and CBCT radiographic techniques in relation to the presence or absence of the second root canal in the MB roots of maxillary molars.

In this sense, in order to overcome the problems of the 2D images provided by the radiographs, the CBCT images provide a visualization of high-resolution images in multiple planes while eliminating superimposition of surrounding structures (Mirmohammadi et al., 2015; Torres et al., 2015). In the present study, by the CBCT images that were used, voxels are isotropic, which means that 3D objects can be measured in three dimensions with relatively good accuracy, detecting 67% of MB2 in the sample (Baratto Filho et al., 2009; Torres et al., 2015). The superiority of the CBCT was comproved in similar results by Vasundhara; Lashkari (2017) (68.3%). Although, minor occurrences were detected by Baratto Filho et al. (2009) (37,05%).

The agreement analysis in the present study demonstrated a slight concordance of the digital radiography in relation to CBCT, being the superiority of this last one proved by a greater detection of MB2. These findings are supported with results from previous studies comparing the accuracy of this and other methods with CBCT (Machado et al., 2021; Pereira et al., 2021; Ratanajirasut et al., 2018; Torres et al., 2015). Thus, CBCT can be considered a great and reliable option for routine clinical use.

The evaluation method of the internal anatomy through root cross sectioning provides information about root canals next than it is possible to detect clinically (Weine et al., 2012). *Ex vivo* studies on the incidence of MB2 canals revealed a higher detection of MB2 than *in vivo* studies (Baratto Filho et al., 2009; Ratanajirasut et al., 2018). Also, studies using an

operating microscope, clearing technique, or sectioning methodology show higher detection rates than radiographic or CBCT examinations (Weine et al., 2012). Seidberg et al. (1973) identified 33.3% of the 201 teeth studied had a MB2 canal in their *in vivo* study. This increased to 62% in their *ex vivo* study of 100 teeth. However, in the present study, CBCT revealed a greater detection of MB2 canal (67%) compared to the method of sections (55%), although presented fair agreement (0.32; 95%CI: 0.09 to 0.56). The CBCT was already compared with the clearing method, considered, until the advent of micro computed tomography, the gold standard of the *ex vivo* studies. CBCT demonstrated results similar to clearing method (Ordinola-Zapata, 2017).

Furthermore, the CBCT analysis seems be more accurate and precisely then CS, considered control method in the present, because in the fist it was possible to analyze a superior number of slices in which third, even if we have employed microscopic evaluation at last. This methodological detail corroborates even more with the thesis that CBCT presents a high level of reliability in the detection of additional canals in maxillary molars (Gupta et al., 2017; Torres et al., 2015).

4. Conclusion

Under the experimental conditions of this study, it can be concluded that MB2 is present in most of the maxillary molars and CBCT was the most reliable clinical technique used to identify MB2 canal. Furthermore, DOM can help in clinical identification of this canal and probably would improve the canal treatment. The authors suggest that further studies can be carried out comparing all the methods that can be used to improve the chances of detecting this accessory canal by clinicians and specialists.

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