# Age-related Variations at the Cementodentinal Junction: An Ex Vivo Study

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**Objective: The objective of this study was to determine the age-related anatomical changes that take place at the cementodentinal junction (CDJ).**

**Methods: Eighty-four teeth were extracted; 42 samples came from patients ranging in age from 18 to 30 years, and 42 came from patients aged from 40 to 60 years. Upper and lower and anterior and posterior teeth were included. Longitudinal slices were made, and 1% toluidine blue was used to stain all the samples prior to microscopic examination. Anatomical landmarks (apical foramen [AF], apical vertex, and cementoenamel junction) in the apical third were identified, and a pre-calibrated software package was employed to take digital measurements. Statistical analysis was performed by means of the Wilcoxon rank-sum test.**

**Results: The data obtained showed that there were anatomical variations in the apical third in the older patients and that these changes were related to the age of the patient. Narrower root canals and smaller CDJ diameters were found in older patients' samples.**

**Conclusion: The results of this study suggest that instrumentation and obturation should take place 1 mm from the AF in older patients, and not 0.5 mm, as is usually recommended. [*P R Health Sci J 2021;40:75-80*]**

*Key words: Aging, Apical anatomy, Cementodentinal junction*

Endodontic therapy represents a challenge. Shaping, cleaning, and filling the root canal system requires not only skillful hands but also profound knowledge of the anatomy and internal structure of each tooth. Several factors should be considered before performing endodontic treatment. To ensure a positive prognosis for a proposed root-canal treatment, care must be taken to both select a viable case and perform a thorough diagnosis. Radiographs are an important aid prior to and during endodontic treatment; they provide valuable information concerning root canal anatomy and morphology (1). However, data provided by x-ray may not be sufficient to clarify all variations that the root canal system might present, meaning that a deeper understanding of the internal anatomy might be necessary.

According to Simon (2), 4 discrete landmarks can be found in the apical region of the root. They are the apical constriction (AC), apical foramen (AF), apex (anatomic and radiographic), and cementodentinal junction (CDJ). Clinicians consider the AF to be a landmark of the apical portion, and it is used as an endpoint for root canal treatment (3). It has been previously stated that optimal healing conditions occur when the obturation material and the apical tissues have minimal contact and when the treatment is terminated at the CDJ (4). The AC should be a reference for determining working length and must be as close to the CDJ as possible, according to the quality guidelines of the European Society of Endodontology (5).

Yuri Kuttler (1955) stated that "anatomy . . . is the foundation of the art and science of healing." This author performed a study in which 268 extracted teeth were observed (by microscope) at their apices, and measurements of these apices were taken to obtain relevant data that could be applied clinically (6). Kuttler's results revealed that the distance between the AF and the AC ranged from 0.4 to 1.2 mm and that the distance of the AC from that of the root apex ranged from 0.5 to 1.01 mm. The AC was reported to be located in dentin or at the CDJ and, less frequently, at the cementum. Hassanien and colleagues (7) performed a study on mandibular premolars, and the authors' results demonstrated that the AC was always found to be located coronal to the CDJ. This means that the locations of the AC and CDJ might vary and not always match, rendering these landmarks not reliable for endodontic therapy at the apical

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vertex (AV), which is the endpoint of the root canal system and the vertex of the tooth. Most importantly, these landmarks are mainly determined histologically and can be interpreted differently by the specialist when observed by radiography.

Particular attention should be paid to analyses of the interrelationship of the dentin and cementum, mineralized tissues involved in the composition of the tooth and which form its apical structure. Measurement studies of human dentition have revealed that the shape and contours of the foramen are affected by dynamic variables. On the external apical surface, layers of cementum fold into the foraminal opening and extend differing lengths—which lengths are determined by such factors as age, occlusal interferences, and injuries—in a coronal direction (8). Of the previously mentioned factors, age is one of the most important, as it is a key element in the positions of the AF, apex, and CDJ (6). Because of the age-related increase associated with the apical cementum, the foramen's center experiences a progressive deviation from the apical center of the root.

Vertucci (1984) determined the number and types of root canals, the number and location of lateral canals and apical foramens, and the frequency of apical deltas utilizing a sample of 100 mandibular incisors, lateral incisors, and canines, identifying up to 8 different configurations of the root canal system (9).

None of the previously mentioned studies has been updated, and the need to understand the apical anatomy of the root canal system is a sure path toward clinical success and better prognosis in endodontic therapy. This is because no one optimal endpoint has been established as the ideal, and variations in the internal anatomy have been widely reported (10–12).

The objective of this study was to determine the age-related anatomical changes that take place at the cementodentinal junction (CDJ). The tested hypothesis was as follows: CDJ to AF and CDJ to AV distances are lower in young patients than they are in those who are from 40 to 60 years old, with those distances being determined with digital measurement techniques.

## Methods

An ex vivo transversal study was carried out. This study was approved by the institutional ethics committee (CEI-FE-010-015). A total of 84 extracted teeth were selected. A visual inspection was carried out on each sample prior to its inclusion. Briefly, the extracted teeth were rinsed with saline solution, left in a container with 1% sodium hypochlorite for 3 days, and, finally, sonicated to eliminate any residues and soft tissue. The teeth were then stored in 10% buffered formalin until they were processed. The samples were obtained from 2 groups of teeth, with the ages of the patients ranging from 18 to 30 years (group A) and 40 to 60 years (group B). The selection criteria included teeth extracted from patients ranging in age from 18 to 60 years, with no history of periapical lesions and no internal or external resorption apparent on radiography. The exclusion criteria included teeth with incomplete root development, a previous diagnosis of pulpitis or necrosis, and fused roots.

The sample size was determined to find a 40% CDJ to AF and CDJ to AV distance reduction between groups. The formula to determine sample size (based on Cohen's d, as previously revealed) was as follows (13):

$$n = \frac{2\left(Z_\alpha + Z_\beta\right)^2 * S^2}{d^2}$$

In the above formula, $Z\alpha$ is the z-value distribution, with a 95% type I error, $Z\beta$ is the z-value distribution with an 80% type II error. S2 is the variability of the distances, based on ElAyouti et al.'s of 550 μm, considering all the canals evaluated in their article (14). And d (Cohen's delta) was the expected value of the difference between groups. In the present study, the difference was assumed to be 40% of the distance as measured by the ElAyouti study; that being the case, then the expected difference between groups was 380 μm. The sample size needed to find this difference was 33 roots per group; the sample size was increased based on the failure of some of the samples.

At this point, the root was selected from each tooth, yielding a total of 42 roots per group (7 lower incisors, 7 lower premolars, 7 lower distal roots from lower molars, 7 upper incisors, 7 upper premolars, and 7 palatal roots from upper molars). The remnants of the teeth were discarded according to the Official Mexican Norm (NOM-087-ECOL-SSA1-2002), which details protocols for dealing with biological waste (15).

### Sample processing

The teeth were mounted on an acrylic block, allowing better sample handling; they were first decoronated, and then each selected root was sectioned by using a 15h 4" diamond disc (Buehler, Lake Bluff, IL, USA) mounted on a slow-speed saw (IsoMet, Buehler). The AF was identified by a stereoscopic microscope (EZ4 HD, Leica, Germany). Longitudinal slices 600 μm thick were obtained and observed on the stereoscopic microscope. Only slices with visible cement, dentin, root canals, and foramina were included in the study (Figure 1).

### Sample staining

Sectioned root apexes were rinsed with 1% phosphate-buffered saline (PBS) solution at 4°C. They were then stored in plastic containers with 1% toluidine blue stain (HY740601000, Hycel, Mexico). Samples were left for 24 h at 4°C to achieve deep penetration of the staining solution. Finally, the samples were rinsed with 1% PBS and examined under a stereoscopic microscope.

### Sample examination

Microscopic examinations were carried out with a stereoscopic microscope (Leica, EZ4 HD, Germany) at 30X. The optimal setup for the examinations was achieved with the settings at max-contrast and mid-brightness. The CDJ, AF, and AV were identified and the images taken using the same settings. The unit

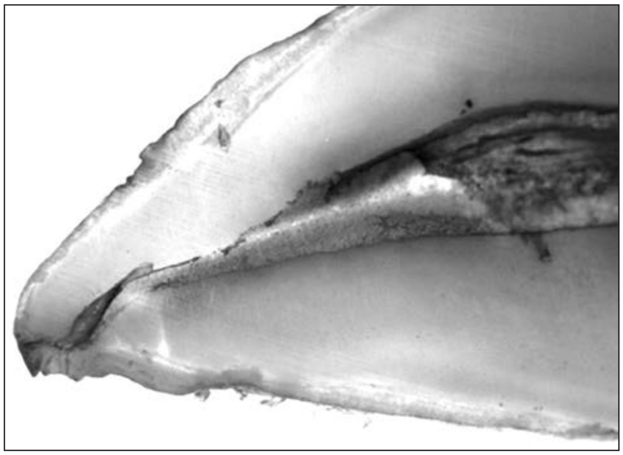

**Figure 1**. Slice effected following the longitudinal axis.

of measurement used for the images was the micron (Figure 2). The calibration process included the identification of 20 random sets of samples. Two independent evaluators determined the specific locations of CDJ, AF, and AV and their findings were compared with those of an experienced evaluator; concordance between the observers was determined with Cohen's kappa test. Based on the kappa values the agreements between observers were 0.9, 0.95, and 0.89 for the AV, CDJ, and AF structures, respectively.

### Anatomical measurements

The images were analyzed using the Leica LAS EZ software (ver. 1.8.1) to conduct all the measurements. CDJ diameter, AF diameter, distance from mid-AF to mid-CDJ, and distance from mid-AF to mid-AV were digitally measured (Figure 3).

### Statistical analysis

Descriptive statistics was used for each variable. The Wilcoxon rank-sum test was employed for this study ($p<0.05$). R software, ver. 3.2, was utilized to perform the test. Data normality was evaluated using the Shapiro–Wilks test; the data distribution was found to be different from normal ($p<0.001$). The CDJ, AF diameters and CDJ to FA, CDJ to AV distances between groups were evaluated with the Wilcoxon rank-sum.

## Results

Digital analysis of the digital images found an average CDJ diameter of 319.6 (± 36.82) microns for the samples in group A and of 292.6 (± 37.45) microns for those in group B. Regarding AF diameter, the average for the group A samples was 508.8 (± 43.68) microns and 677 (± 106.03) microns for the group B samples. Microscopic examination revealed that the AF was never located at the AV (0% match). CDJ to FA distance averaged 582.6 (± 61.03) microns for the samples in group A and 727.3 (± 168.76) microns for the group B samples. VA to FA distance averaged 499 (± 48.66) microns for the samples in group A and 609 (± 117.69) microns for those in group B (Table 1). All the comparisons between groups were statistically significant ($p<0.001$).

### Discussion

The examination and analysis of the apical third using a stereoscopic microscope permits a better understanding of root canal anatomy and provides relevant data for the clinician. Profound knowledge of the root canal system is required to achieve a successful endodontic treatment (7,12,16). The CDJ has previously been mentioned as the ideal landmark for instrumentation in endodontic therapy (2). Kuttler reported narrower CDJ measurements in older patients (268 microns), which finding is in agreement with the results obtained in this study (292.6 microns) (6). However, Stein and Corcoran conducted a study on 47 samples and found that the AP was wider in older patients but that the CDJ remained the same (17). The latter finding might be linked to the thickness of the slices (500 microns) studied, which thickness might not be sufficient for the clinician to obtain a clear view of the structure of the root canal, leading to a misinterpretation of the measurements.

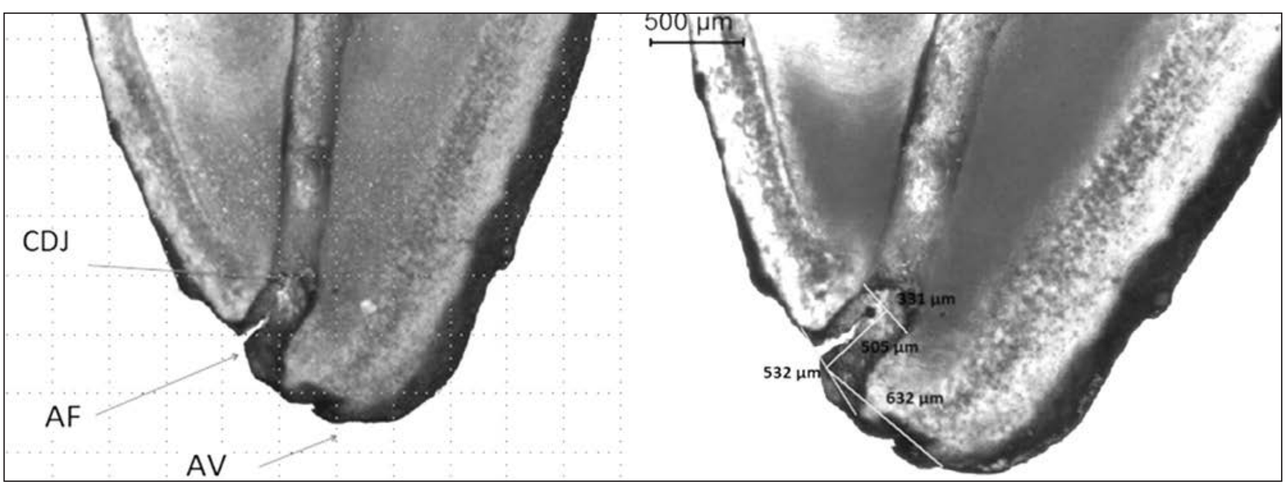


**Figure 2**. Identification of the anatomical landmarks after staining was performed and measurements were taken (digitally).

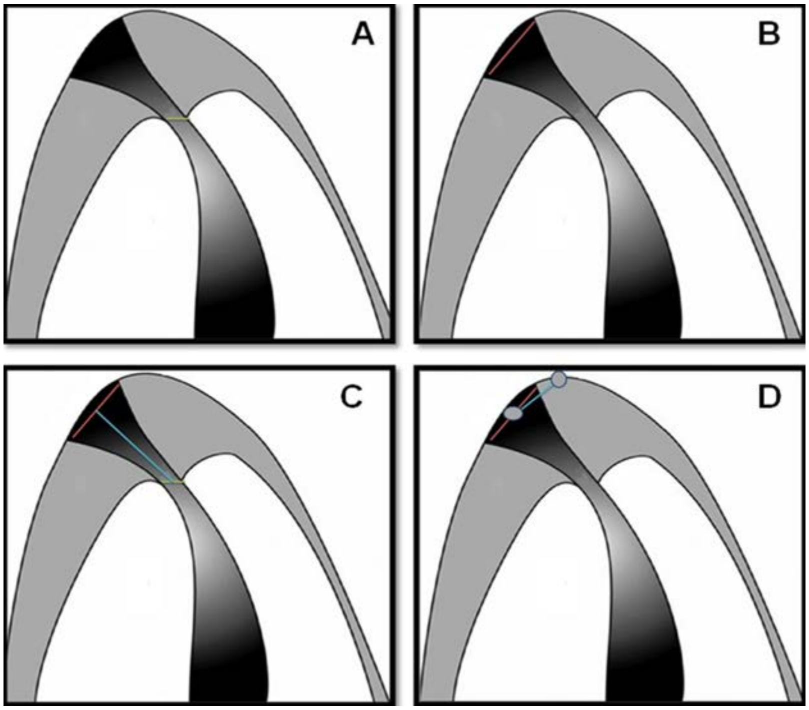


**Figure 3**. Different anatomical measurements. A. Cementodentinal junction (CDJ); B. apical foramen (AF); C. distance from mid-FA to mid-CDJ; D. distance from mid-AF to mid-apical vertex (AV).

According to Briseño and colleagues (18), despite the age of the patient, the CDJ possesses a diameter that is approximately equal to that of the tip of a #30-#35 K-file, meaning that instrumentation must be performed with no less than a #30 K-file to ensure root canal cleaning. Such is not the same for the palatal and distal canals, where a #35 K-file can easily achieve working length when used as the first instrument. Additionally, scouting canals must be made with a K-file smaller in diameter than the CDJ, meaning that a #25 K-file or higher should not be used for such a purpose. In addition, if any resistance is encountered, it might be due to a curved canal and not a narrow CDJ. This study showed CDJ diameters that clearly were wider than a #25 K-file, in agreement with the concept described previously and suggesting that finishing instrumentation with a #25 K-file could result in under instrumented canals.

In the present study, the AF was not found at the AV in 100% of the roots. The average diameter in the group A samples was 508.8 microns, and 677 microns in the group B samples, which is not in agreement with the results obtained by either Kuttler (6) or Briseño (18). The average diameter of the AF in the samples in group A was also smaller than that in the group B samples, which difference is similar to what was reported by Kuttler (6). This can be explained by the fact that cementum apposition increases over the years, causing the AF to increase in size, as well. This also explains why the hourglass-shaped foramen is found more frequently in older patients than it is in their younger counterparts. It is worth considering that fillings extended to the radiographic apex, especially in older patients, are indeed underfilled because these cases are not usually instrumented up to a #70 K-file. A few articles have reported on the distance between CDJ and AF. Our knowledge is based on Kuttler's data, which reported an average distance from the CDJ to the AF of 524 microns in younger patients and of 659 microns in older patients (6). Compared to Kuttler's measures, the average CDJ to AF distances found in this study were longer for both younger (18–30 years of age) and older (40–60 years of age) patients, being 582.6 microns for the former and 727.3 microns for the latter (Table 1). This discrepancy might be due to several things that happened during the evaluation. Longitudinal slices stained with 1% toluidine blue and examined by stereoscopic microscopy allowed the clear identification of the CDJ, and measurement by specialized software provided more accurate numbers than those obtained by Kuttler (6). These variations in the measurements could represent important clinical considerations, especially for older patients (40–60 years of age), suggesting new parameters in working length that should be considered when performing root canal treatments. The results of this study are similar to those obtained by Briseño and colleagues (18), who examined 1,097 teeth by means of a computer-aided stereomicroscope, obtaining average measurements of 0.86 to 1 mm. Although more in-depth research is required, most studies have revealed similar results when it comes to older patients, for whom these measurements are usually higher than those obtained for younger patients. Though the results in our study are not definitive in determining where instrumentation and filling

**Table 1**. Statistic data of the measurements made at different anatomical landmarks.

| AGE | CDJ Diameter | AF Diameter | CDJ to AF Distance | AF to AV Distance |
|---|---|---|---|---|
| 18–30 years (group A) | MEAN ± SD<br>319.6 ± 36.82<br>MDN (RANGE)<br>310 (259–423) | MEAN ± SD<br>508.8 ± 43.68<br>MDN (RANGE)<br>508 (367–612) | MEAN ± SD<br>582.6 ± 61.03<br>MDN (RANGE)<br>595.5 (363–691) | MEAN ± SD<br>499 ± 48.66<br>MDN (RANGE)<br>495 (341–632) |
| 40–60 years (group B) | MEAN ± SD<br>292.6 ± 37.45<br>MDN (RANGE)<br>290.5 (221–418) | MEAN ± SD<br>677 ± 106.03<br>MDN (RANGE)<br>691 (400–856) | MEAN ± SD<br>727.3 ± 168.76<br>MDN (RANGE)<br>761.5 (289–1166) | MEAN ± SD<br>609 ± 117.69<br>MDN (RANGE)<br>607 (314–901) |
| P value | <0.001 | <0.001 | <0.001 | <0.001 |

CDJ: cementodentinal junction; AF: apical foramen; CDJ to AF: cementodentinal junction to apical foramen; AF to AV: apical foramen to apical vertex; MDN: median; SD: standard deviation. All measurements are reported in micrometers.

should be performed, they do suggest that, for older patients, this procedure should take place 1 mm from the AF, rather than 0.5 mm, which is the is the usual recommendation.

Stabholz et al. summarized factors influencing success and failure in endodontics, based on retrospective studies, finding no conclusive evidence that the number of appointments, use of intracanal medication between appointments, or obturation technique had any impact on the success of a given treatment (19). However, all the studies that Stabholz and colleagues reviewed agreed that the extension of the filling is the main factor influencing the outcome of an individual root canal treatment, yet the best landmark for root canal filling remains controversial and undefined. It is currently accepted that extending the filling materials beyond the apical limits into periapical tissues causes irritation and, consequently, delays healing. Nonetheless, extrusion of the endodontic sealer is accepted and is not considered to affect the prognosis of the outcome of endodontic therapy (20). Currently, most clinicians opt to perform biological root canal treatment by cleaning, shaping, and filling within the root limits.

This study determined that the location of the CDJ strongly correlates with the age of the patient, suggesting that the overall knowledge of apical anatomy needs generally to be reviewed and updated so that reliable landmarks can be identified for obturation in endodontic therapy. The measurements obtained in this study of the distance from AV to AF agree with those reported by Kuttler (1955), who found that this distance is longer in older patients (6). Complementary to these data are the findings of Dummer and colleagues, who previously reported that there was a greater increase of these measurements in posterior teeth than there was in anterior teeth (21). The average distance was 609 microns in older patients (40–60 years of age) and 499 microns in young patients (aged 18–30 years). These data are similar to those in Burch's results, in which the measurements averaged 590 microns (22).

The closure of the root end with cementum, while both possible and desirable, is necessary for the health and function of the apical periodontium. Aging is the most important factor in terms of the closure of a root canal by the cementum, and this cementum apposition increases the distance between the CDJ and the foramen (23). The aging process of the tooth causes cement deposition; consequently, the distance of the AF from the AV increases. Such an increase is variable and represents relevant data to the clinician, because most cases are guided by the radiographic vertex. Nevertheless, such may lead to an error—in that the AF is not always located at the apical vertex—and this occurs frequently in older patients. We suggest, based on the data obtained in this study, that clinicians use an apex locator to obtain a reliable measurement when performing endodontic therapy. Radiography is a complementary tool in endodontics, one that can be used to aid in diagnosis or to assess the quality of the obturation. It should not, however, be considered for establishing a landmark when determining working length.

Due to the presence of sharp curvatures, variable anatomy, and reduced thickness, we opted to work with palatal roots, because they present fewer anatomical variables and the thickness allows for better manipulation. We chose 1% toluidine blue as the dying solution because it is possible to stain cement and dentin at different intensities, as previously reported, allowing the operator to easily identify the anatomical structures when performing stereoscopic or microscopic examination (24).

Apical constriction was not found in all the samples. These results are similar to those reported by Dummer and colleagues (21). That being the case, apical constriction was not considered in this study. It is also noteworthy that the methodology employed in this study might not show the narrower portion of the root canal, because inner dimensions vary depending on the plane in which the slices were made, especially in ovoid root canals. In contrast, another study exhibited 100% incidence on employing microtomography (14).

In all the samples (100%) in the present study, the region of the apical third was seen to be shaped like an hourglass. Dummer and colleagues (21) reported that only 39% and 52%, respectively, of root canals of the anterior teeth and premolars in their study sample had that shape. more frequently, Dummer and team found them to be ribbon shaped; these contrasting results might have been caused by the methodology utilized in each study, which emphasizes the difficulty of visualizing and identifying apical anatomy. Deep knowledge of a given tooth's apical anatomy is not achievable with radiography; therefore, microscopic examination in vitro is necessary to obtain reliable information regarding complex variations. The results obtained in this study suggest that there is a need to perform newer studies of apical anatomy and emphasize the importance of knowing it and clearly defining appropriate landmarks for endodontic therapy. Data obtained clinically suggest that instrumentation and obturation be performed at least 1 mm from the apical foramen in older patients and not 0.5 mm, as is normally the case.

## Conclusions

The data obtained revealed both anatomical variations at the apical third in older patients and showed how these changes are related to the age of the patient. In this regard, narrower root canals and smaller CDJ diameters were found in the samples of older patients, suggesting the need to use an apex locator rather than radiography to establish a reliable termination endpoint when performing root canal treatment on them. The findings in this study suggest that instrumentation and obturation be performed 1 mm from the AF in older patients and not 0.5 mm, as is usually recommended.

## Resumen

Objetivo: El objetivo de este estudio fue determinar cambios anatómicos relacionados con la edad en la unión cementodentinaria (UCD). Métodos: Se extrajeron 84 dientes; 42 de pacientes con edades entre 18 y 30 años, y 42 de pacientes con edades entre 40 y 60 años. Se incluyeron dientes superiores, inferiores, anteriores y posteriores. Se hicieron cortes longitudinales y se usó azul de toluidina al 1% para teñir las muestras, previo a la observación microscópica. Se identificaron puntos anatómicos (foramen apical [FA], vértice apical y unión esmalte-cemento) en tercio apical y se empleó un software precalibrado para realizar mediciones digitalmente. El análisis estadístico se realizó mediante la prueba de Wilcoxon. Resultados: Los datos obtenidos mostraron variaciones anatómicas en tercio apical en pacientes mayores y cómo estos cambios están relacionados con la edad. Se encontraron conductos más estrechos y diámetros de UCD menores en muestras de pacientes mayores. Conclusiones: Los resultados de este estudio sugieren que la instrumentación y obturación debe realizarse a 1 mm del FA en los pacientes mayores, y no a 0,5 mm, como suele recomendarse.

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