Exploration of Middle Mesial and Middle Distal Canals in Lower First Molars in an Egyptian population Sample Using Different Methods: An (In vitro-In vivo) study.

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Dedication

A special thanks to my family. Words cannot express how grateful I am to My **Father, Mother, Brothers** for all of their support that they have made for me.

Finally, I must express my very profound gratitude to **my dear: Eng. / Gehad Taha "my best assistant"** and to my little princess **Razan** for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them.

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List of Appreviation

no.	Appreviation	Discerption
1	DOM	dental operating microscope
2	CBCT	cone-beam computed tomography
3	MMC	middle mesial canal
4	MDC	middle distal canal
5	AAE	American Association of Endodontists
6	AAOMR	the American Academy of Oral and Maxillofacial Radiology
7	µCT	micro-computed tomographic
8	NaOCl	sodium hypochlorite
9	CEJ	cementoenamel junction
10	r	Pearson correlation value

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Introduction

One of the most common causes of endodontic failure is a missed root canal anatomy; therefore better awareness of the root canal anatomy is mandatory to improve the success rate of endodontic treatment (1-5) Besides, the clinician should be aware of the possible variations in canal anatomy that may be encountered (6,7).

Many techniques were used to examine the root canal anatomy such as sectioning, decalcification, radiographs, clearing/staining, magnification using (loupes& dental operating microscope DOM) and cone-beam computed tomography CBCT (8-13).

Magnification of the operating field allows for a better vision besides increasing the accuracy of the endodontic field. It was proven that the high levels of magnification increase the amount of visual information required for the detection of extra-canals (14,15).

Additionally, cone beam computed tomography "CBCT" is an extra-oral imaging system specifically designed for three-dimensional imaging of the oral and maxillofacial structures. Most of the limitations associated with conventional radiography like compression of a three-dimensional object into a two-dimensional image, image distortion and anatomic superimposition were overcome by CBCT (16,17).

Recently, magnification in-conjunction with three-dimensional imaging of the root canal system played an important role in the detection of teeth with extra-canals such as the middle mesial canal (MMC) and the middle distal canal (MDC) in mandibular first molars(18, 19).

The mandibular molar is the first permanent molar that erupts into the oral cavity and it is most often tooth requiring endodontic therapy. Therefore, the knowledge of its common variations (extra canals "MMC&MDC") should be kept in mind to avoid the failure of endodontic treatment (7).

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Very little research have been done to detect both of MMC&MDC in mandibular first molar in an Egyptian population, so this study was done to explore the prevalence MMC&MDC in mandibular first molars in an Egyptian population sample using different methods (cone beam computed tomography "CBCT", dental operating microscope "DOM" and clearing technique). The null hypothesis stated that there is no difference among the methods used to explore both of the middle mesial and the middle distal canals.

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Review of literature

Section outline:

2.1. The complexity of root canal morphology.

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Review of literature:

2.1. The complexity of root canal morphology.

No one can deny that the complexity of root canal anatomy in terms of the presence of extra roots and root canals with lateral and accessory canals has a great significance on the endodontic outcome(5,20,21,22). Furthermore, the success of endodontic therapy depends on the ability of the clinician to locate, clean, shape and obturate all root canals effectively (23). As the root canal system is a complex one, the course of the canal from the orifice to the apex shows different varieties of configuration from teeth to teeth in different as well as in the same individual (7,24).

2.2. Variations in canal configuration in a mandibular first molar.

The mandibular first molar is the first posterior tooth that erupts and is the tooth that most often requires root canal treatment. Often it has two roots but occasionally, it has three with two, three or more canals in the mesial root and one, two, three or more canals in the distal root (25).

Regarding mesial root in 90%, they remain separate as far as the foramen; in the remaining 10%, they join at a common foramen. The canals of the mesial root take a more curved course with a mesial orientation immediately below the orifice and then distal in the rest of the root canal (26). Previous in vitro and in vivo study have indicated that mandibular molars can have more than three root canals; mesial and distal roots with three canals each or molar with 5, 6, or more canals(7). Ahmed et al (27) in 2007 studied the root and root canal morphology of permanent mandibular molars in a Sudanese population and he found that the prevalence of three mesial canals was 4 %. Ballullaya et al (7) in 2013 reviewed the presence of MMC and MDC in mandibular first molar in different populations, and he found that the prevalence of MMC in mandibular first molar ranging from 1-15% and MDC in mandibular first molar is 1.7 %. Azim et al. (28) in 2015 reported that prevalence of MMCs in mandibular molars after guided troughing under high magnification in Columbian and USA patients and found that the MMC was

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present in 46.2% of mandibular molars. Filpo-Perez et al. [29] in 2015 evaluated the morphologic aspects of the root canal anatomy of the distal root of a mandibular first molar using micro-computed tomographic analysis in Brazilian population and he found The distal roots of the mandibular first molars showed a high prevalence of single root canals as follow 76% of the distal roots had a single root canal, while in 13% of the roots were 2 root canals, in 8% of the roots were 3 root canals and finally 3% of the distal roots were containing 4 root canals.

2.3. Visualization methods of root canal system:

Several methods have been suggested to study the root canal system configuration and the complexity of the first mandibular molar.

2.3.1. Historical visualization methods.:

These old methods were worked to reconstruct the root canal system. Preiswerk [30] (1901), employed Wood's metal; an alloy that melts at a low temperature, which flows down into the root canals, and Following heat dissipation, the tooth is dissolved, and exposing the internal metal cast of the pulp cavity. Only a few years later, Fischer [31] (1907), used a solution of celluloid dissolved in acetone, instead of Wood's metal, to visualize the pulp cavity. The methods used by Preiswerk and Fischer can still be successfully used to reproduce the shape of root canals, but, due to the necessary corrosion of the tooth surrounding the cast, these techniques do not provide a precise impression of the relation between the pulp cavity and the mineralized part of the tooth. For this problem, Okumura [32] (1911), used grinding sections that allowed a more direct investigation of the relation between the external and internal structure of the tooth which created numerous sections from all permanent teeth to describe and measure several important endodontic parameters, including the position of the floor of the pulp cavity, the thickness of the pulp cavity wall, the distance from the cervical line to the furcation area, the number, size, and shape of root apical ramifications, as well as the horizontal sectional variations at the level of the root canal orifice and apical area. However, because the study was presented in

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Japanese only, Okumura's work was not met with much international recognition and the approach was therefore not adopted by other endodontic researchers. Two years after Okumura's study, Adloff [33](1913), improved Preiswerk's approach by first injecting molten metal into root canals, then rendering the studied teeth transparent to investigate the relationship between the pulp cavity and the remaining tissue. Moral [34,35] (1914, 1915), reported another method, where hard tissues were also made transparent, but where the pulp cavity was filled with Indian ink instead of Wood's metal. Hess [36] (1917), conducted experiments aimed at reproducing the results obtained by Preiswerk and Adloff. However, his trials largely failed due to cracks in the dentine that formed during the heating process. Only a few years later, Hess [37] (1925), studied the anatomy of different root canals from a sample comprising 2800 permanent human teeth, which he succeeded in reconstructing the root canal shape on such a large scale by using a novel approach that involved filling the teeth with vulcanized rubber. To complement his findings, he made grinding sections of selected teeth to illustrate the fine structure of the root canals in their apical region. In addition to the invasive techniques employed by these early researchers, X-ray imaging was soon found to constitute a valuable approach for exploring the internal structure of the human tooth. In this study, Mueller [38] (1933), used X-ray imaging to investigate extracted teeth filled with gutta-percha. Instead of relying on X-ray imaging, Hirano et al. [39] (1958), further advancing the invasive techniques employed by previous workers, used Indian ink to visualize the root canal shape but instead rendered the teeth transparent using silicone oil. A similar approach was later employed by Barker et al. [40] (1969), Carns et al. [41] (1973), using epoxy resin replicas. However, because these techniques were complex and time-consuming, only a few subsequent studies pursued these approaches. A significant step forward was later made by Vertucci et al. [42] (1974), documented a broad morphological variation in the root canals of 200 maxillary second premolars using hematoxylin to stain the fresh pulp tissue, while the teeth were

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completely cleared using a liquid plastic resin following dehydration in alcohol. Since then, this approach has become a standard method in endodontic research, and numerous reports have been published that elucidate root canal systems using this so-called "clearing technique" [43-46].

In the previous methods, a considerable range of techniques has been successfully employed to visualize the anatomy of human teeth. However, these methods often required the partial or even full destruction of the studied samples and were not digitized, thus rendering the distribution of the raw data difficult.

2.3.2.Invasive visualization methods.

2.3.2.1. Modifying the access cavity.

The term access cavity refers to the part of the cavity from the occlusal table to the canal orifice. However, its design, which includes location, shape, and size, depends on the position of the canal orifices as well as the position and curvature of the canal throughout its entire length. Sufficient tooth structure should be removed to allow instruments to be placed easily into the orifice of each canal without interfering with overhanging walls during root canal preparation. The orifice of each canal must be visible and easily accessible for instrument placement. If extra roots and/or canals are suspected at the diagnosis stage, the access cavity outline form should be modified [47]. To prepare a proper access cavity, the practitioner must have a thorough knowledge of the pulp chamber anatomy. The pre-operative radiographies should be carefully examined to determine the anatomy of the individual tooth that is about to be treated. The use of a specially-designed instrument such as a DG16 endodontic probe significantly facilitates the inspection of the pulp chamber floor and the discovery of canal orifices once the pulp chamber is exposed [48,49].

The anatomical laws of Krasner and Rankow are useful general anatomical landmarks (independent of the crown anatomy) that may help the clinicians to localize the orifices of canals that are not readily visible. Therefore, upon opening the pulp chamber, clinicians should consider these laws [50]. As with all cavities

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and crown preparations, endodontic access cavities should be specifically designed for each tooth and each patient. Parameters such as the degree of canal curvature, the position of the canal apex relative to its cusp tip, canal length, the degree of calcification, size and shape of the canals plus the position of the tooth in the jaw, all determine the specific design of an access cavity [47].

For example, in mandibular molars, the chamber is usually triangular to a square in shape. The access opening is triangular to slightly square on the occlusal surface, and its preparation should be distal to the mesial marginal ridge and primarily within the mesial half of the occlusal surface, keeping in mind that the distal extension of the access opening should extend into the distal half of the tooth. The access cavity for the mandibular first molar is typically trapezoid or rhomboid regardless of the number of canals present. When four or more canals are present, the corners of the trapezoid or rhombus should correspond to the positions of the main orifices. Mesially, the access does not need to invade the marginal ridge. The distal extension must allow straight-line access to the distal canal(s). The buccal wall forms a straight connection between the MB and DB orifices, and the lingual wall connects the ML and DL orifices without bowing [51, 52].

Middle mesial (MMC) canals are located between mesiobuccal and mesiolingual canals of mandibular molars with the incidence of 1-15% for mandibular first molars and 10% for mandibular second molars. In almost all of the reported clinical cases, this canal joined the mesio-buccal or mesio-lingual canal in the apical third [53].

2.3.2.2.Transillumination:

Transillumination is the technique of sample illumination by the transmission of light through the sample. It is used in a variety of imaging methods. In medicine, transillumination generally refers to the transmission of light through tissues of the body. In dentistry, bright transilluminated light through the tooth can highlight dental caries, show signs of dental trauma such as coronal fractures

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and assist in locating the missing and calcified canals. It has been suggested that transillumination of the cervical area of a heavily restored tooth can greatly improve visibility and reveal landmarks that otherwise remain invisible to the unaided eye. Transillumination can also aid in detecting calcified canals [54].

2.3.2.3. Ultrasonic scouting of canal:

The use of ultrasonic devices in endodontics has enhanced the treatment and represents an important adjunct when managing difficult cases. The devices have become increasingly more useful in applications such as gaining access to orifices, cleaning, and shaping, filling the canals, removal of intracanal materials and obstructions, and during endodontic surgery [55]. The use of ultrasonic tips with abrasive coatings helps remove dentine conservatively. The working end of these tips is typically about 10-times smaller than the smallest available round burs and consequently, they can be used on the walls and/or floor of the pulp chamber to look for canal orifices. The use of such tips eliminates the bulky heads of conventional handpieces, which often obstruct vision, and they allow this chasing to be carried out under direct vision. Any use of instruments on the floor of the pulp chamber should only be carried out under direct vision because of the risk of perforation [55, 56]. Ultrasonic devices are used by some endodontists for this purpose although the majority of them appear to prefer the use of burs and endodontic explorer [55]. However, the use of ultrasonic tips may be more conservative. Yoshioka et al. also demonstrated that ultrasonic tips were effective in detecting the presence of accessory canals [57]. Karapinar-Kazandag et al. showed that the combined use of a microscope and ultrasonic devices increase the detection of middle mesial canals in mandibular first and second permanent molars, they showed that after locating the main three or four canals, the middle mesial canal was investigated in all teeth firstly without microscopy, then with the aid of a microscope and finally with the combined use of a microscope and ultrasonics; with these techniques, the middle mesial canal was detected [58]. Coelho de Carvalho et al [59] revealed that when microscopy and ultrasonics were

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used, 50 more canals could be visualized, representing a 7.8% increase in the total number of located canals.

2.3.2.4.Magnification:

For a long time, microscopes have been used in various medical specialties [60]. The reasons for their introduction to endodontics were enhanced visibility and lighting. Carr et al. [61] stated that the microscope brings the practitioner right onto the pulp chamber floor and brings minute details into clear view. Lighting is significantly improved because the light of a microscope is parallel to the line of sight and will provide two to three times the light of a surgical headlamp

The use of an operating microscope removes some of the guesswork that existed previously in many areas of endodontic treatment. Michealides [62], stated that the enhanced illumination and visibility enables endodontists to improve the predictability of their procedures. According to Khayat, one of the advantages of using a microscope is to increase the possibility of locating calcified and additional canals [63]. Searching for calcified canals includes the use of the endodontic explorer, troughing with burs or ultrasonic tips and close visual inspection of the root anatomy, which gives clues to the location of the canals. One of the dangers in searching for calcified canals is the possibility of perforation. Using a microscope can give intimate detail of an area that otherwise would be under-illuminated and under-magnified, requiring guesswork and great caution [64, 65].

In the retrospective study, Nosrat et al [66] evaluated the incidence of middle mesial canals in mandibular first and second molars using the dental operating microscope and found that, with using magnification and careful tactile search techniques, the incidence of middle mesial canals in mandibular molars was to be higher than previously reported. Using the operating microscope is key to locating and negotiating extra canals as middle mesial canals. In vivo studies, Azim et al. discussed the prevalence of middle mesial canals in root canal systems of mandibular molars after guided troughing under high magnification and he

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concluded that the middle mesial canal was present in 46.2% of mandibular molars. High magnification and troughing appeared to be determining factors in accessing the middle mesial canal [(67)].

2.3.3. Non-invasive visualization methods; "Radiographic techniques":

- **Conventional radiography.**
- **Magnetic resonance imaging**
- **Computed tomography**
- **Spiral computed tomography**
- **Cone-beam computed tomography**
- **Micro-computed tomography**

- **Conventional radiography:**

Although periapical radiographies are two-dimensional images of the three-dimensional root canal system, their interpretation reveals external and internal anatomical details that suggest the presence of extra canals and/or roots [(68)]. For careful evaluation of the root canal morphology, at least two periapical radiographies should be taken using the parallel technique with either a mesial or distal horizontal tube shift [(69)]. The angled radiographies help to visualize the superimposed roots, allow good visualization of the buccal roots often covered by the palatal root of upper molars, displace the zygomatic process of the maxillary bone that can cover the apices of the molars, and suggest the position (buccal or lingual) of foreign bodies [(69)]. Martinez-Lozano et al.[(70)] examined the effect of x-ray tube inclination on the accuracy of determining the root canal anatomy of premolars and he found varying the horizontal angle, the number of root canals observed in maxillary premolars coincided with the actual number of canals present.

Pre-operative radiographies should be examined carefully; a sudden change in the radiographic density of the pulp space usually indicates an additional canal, whereas a sudden narrowing or even disappearance of the root canal space indicates a bi- or a tri-furcation [(68)]. Friedman et al. [(71)] showed the critical

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importance of pre-operative radiographies in identifying the complex canal morphology.

Post-operative radiographies can also provide valuable information about the presence and position of an extra root and/or canal. If the root filling material is not centered within the root, there may be a missed canal. Hoen and Pink indicated a significant correlation between the asymmetric position of the previous root filling material and the ability to locate untreated root canals (72).

Notwithstanding the above, radiographies may not reveal all canal bifurcations, accessory canals, and apical deltas. Nattress et al. (73) reported that one-third of the canal bifurcations in roots assessed by viewing radiographies taken in the standard bucco-lingual direction were not visible (74). In another study, Bedford et al. showed that the radiographies were not sensitive in assessing the number of the present root canals.

In summary, the information regarding root canal anatomy that is evident radiographically is valuable but also limited, and it should always be integrated with a careful clinical examination. The pre-operative radiographic analysis is critical for endodontics and multiple angled periapical views help to reveal the presence of roots and root canal systems.

- **Magnetic resonance imaging:**

MRI is a non-invasive imaging technique based on the principle of nuclear magnetic resonance (75). The major advantage of this technique for human diagnostics is the absence of any form of ionizing radiation. Furthermore, MRI provides excellent soft tissue contrast without the need for contrast agents. However, hard tissue is not as good in MRI scans as it is in scans made using X-ray-based imaging techniques such as CT. Nonetheless, MRI can be successfully used to visualize tooth anatomy, in particular if specimens are analyzed in a hydrated state. Tymofiyeva et al. (76) provide a review of dental studies using MRI. While clinical (i.e., human) MRI scanners can usually provide isotropic voxel resolutions of about 500 µm, pre-clinical (i.e, small animal) scanners may

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permit the acquisition of isotropic voxel resolutions down to 20 µm, depending on the size of the sample and the magnetic field strength of the scanner[77]. Although a not common practice, endodontic research has already benefitted from using MRI systems. For example, Tymofiyeva et al [76,78] used clinical MRI scanners to visualize dental pulp in vivo at isotropic voxel resolutions down to 112 µm. A comparative study by Gaudino et al. [79] using MRI and different CT techniques concluded that MRI is capable of better characterizing soft tissues and could be applied to the detection of inflammatory or neoplastic pathologies at an early stage.

- **Computed tomography**

In contrast to MRI, 3D data on mineralized tissues can be rapidly and reliably obtained using the X-ray-based imaging technique CT. However, due to the nature of this part of the electromagnetic spectrum, CT methods are invariably associated with varying degrees of ionizing radiation [80]. Originally developed in the early 1970s as a medical imaging technique by Houndsfield [81]. CT has now become available at different levels of resolution [80]. CT can facilitate the assessment of the internal root canal morphology. The advantage of CT scanning over conventional radiography is that it allows the operator to look at multiple slices of tooth roots and their root canal systems [82, 83]. CT images can also help to identify many morphologic variations than panoramic radiographies [84]. Various instruments are being used in endodontic research, in particular, spiral CT, CBCT,and µCT.

- **Spiral computed tomography:**

Some case reports show that spiral CT can be an effective tool for validating internal tooth structures [85,86]. However, the isotropic voxel resolutions that can be obtained are currently limited to about 200 µm, which does not permit the resolution of finer root canal structures [87, 88]. In addition, spiral CT exposes the patient to a significantly higher radiation dose than intraoral or panoramic

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radiography. Consequently, the application of spiral CT scanners in dentistry is limited [89].

- **Cone-beam computed tomography:**

Cone-beam computed tomography (CBCT) is a medical imaging technique where the x-rays are divergent, forming a cone. During dental imaging, the CBCT scanner rotates around the patient's head, obtaining up to nearly 600 distinct images. A single 200-degree rotation over the region of interest acquires a volumetric data set. The scanning software collects the data and reconstructs it, producing what is termed a digital volume composed of three-dimensional voxels of anatomical data that can then be manipulated and visualized with specialized software [82-84] . CBCT scanning has been used in endodontics for the effective evaluation of the root canal morphology [90, 91]. Matherne et al. [92] found that CBCT images always resulted in the identification of a greater number of root canals than digital radiographic images.

Shemesh et al. ([93]) found that CBCT is a good option for identifying root canals and anatomical variations. The latest CBCT apparatuses now permit scans with isotropic voxel resolutions about 75-80 µm [94, 95]. Using a scanner with an equally high resolution, a recent study by Byun et al. [96] showed that CBCT data in conjunction with 3D modeling and additive manufacturing can be successfully used in the treatment of formation anomalies, including internal tooth structures. CBCT has also successfully been used in epidemiological surveys of unusual root morphologies [97, 98]. Torres et al. [99] was used cone-beam computed tomography (CBCT) to characterize mandibular molar root and canal morphology and its variability in Belgian and Chilean population samples and he concluded that CBCT imaging is a useful tool for assessing root and canal anatomy and may assist endodontic specialists in making a diagnosis and planning further treatment. Moreover, the position paper published jointly by the American Association of Endodontists (AAE) and the American Academy of Oral and Maxillofacial Radiology (AAOMR) does not support the routine use of CBCT

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for all cases except when complex root canal anatomy is suspected [100]. This is supported by Reuben et al. [101] who reported that the modified canal staining and clearing technique was as accurate as CBCT in identifying root canal morphology.

- **Micro-computed tomography**

In contrast to CT and CBCT, μCT was originally developed for industrial purposes [80, 102]. Because μCT systems are equipped with a micro-focus X-ray source, scans are made at high isotropic voxel resolutions, sometimes even down to the nanometer scale [80]. However, due to their technical setup, most μCT systems can only be used to study extracted teeth or tissues. Filpo-Perez et al. [29] was evaluated the morphologic aspects of the root canal anatomy of the distal root of a mandibular first molar using micro-computed tomographic analysis and he found that the distal roots of the mandibular first molars showed a high prevalence of single root canals, the prevalence of long oval and flattened canals increased in the coronal direction and 13% of the samples, canal configurations that were not included in Vertucci's configuration system were found.

2.3.4. Other methods:

- **Dyes:**

Various dyes, such as iodine in potassium iodide, ophthalmic dye, or 1% methylene blue can be irrigated into the pulp chambers of the teeth and rinsed thoroughly with water, dried, and visualized. The dye will be absorbed into the orifices, fins, and isthmus areas, and serves to 'roadmap' the anatomy, aiding in the identification of missed canals and fractures. [103, 104].

- **Bubble test/champagne test:**

Sodium hypochlorite flooded into the chamber reacts with the organic tissue or the chelator used. This indicates a positive 'bubble test or champagne test'. This helps in clearing the pulp chamber and improving visibility in the hidden orifices and missed canals. ([103]

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- **Explorer pressure**

An endodontic probe (e.g., DG16, CK17) is a double-ended long probe, which helps in identifying missed canals. It is used to punch through the secondary dentin and calcifications to expose hidden orifices [104].

- **White line test:**

When a shelf of dentin meets the pulpal floor, it forms a groove. During access preparation and exploring with ultrasonic, dust collects in such grooves and forms a visible roadmap that can be followed to locate the missed canals [104].

- **Red line test:**

In vital cases, blood frequently moves into an isthmus area and absorbs into orifices, fins, and isthmuses, which serve to roadmap and aid in the identification of the underlying anatomy [104].

- **Color:**

Orifices appear in a darker color than the surrounding area and can be differentiated and followed to locate the missed canals [104].

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Aim of the study

In vitro study:

Exploration of the middle mesial and the middle distal canals in mandibular first molars in an Egyptian population sample using different methods (cone beam computed tomography "CBCT", dental operating microscope "DOM" and clearing/staining technique).

In vivo study:

Exploration of the middle mesial and the middle distal canals in mandibular first molars in an Egyptian population sample using cone beam computed tomography "CBCT".

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4. Materials and Methods

I. In-vitro study:

4.1. Study design.

4.2. Sample size determination.

4.3. Selection and preparation of the teeth.

4.4. Construction of the plastic molds.

4.5. Holding the teeth inside the plastic molds.

4.6. Methods of exploration of middle mesial(MMC) and middle distal(MDC) canals.

4.6.1. Exploration of the samples using high-resolution cone-beam computed tomography "CBCT" (CBCT exploration method).

4.6.2. Exploration of the samples using the dental operating microscope (DOM exploration method).

4.6.3. Exploration of the cleared samples using the dental operating microscope (clearing/staining exploration method).

4.7. Statistical analysis

II. In-vivo study:

4.8. Study design.

4.9. Sample size determination

4.10. Selection of the CBCT scans of the individuals.

4.11. Cone-beam computed tomography analysis.

4.12. Statistical analysis

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The study approved by the ethics committee of the faculty of dental medicine, Al-Azhar University.

I. In-vitro study:

4.1. Study design:

The in-vitro section of the study was designed as an experimental, non-randomized, controlled trial study in the form of the prevalence of the middle mesial (MMC) and middle distal (MDC) canals using different methods; clearing/staining method as a control method while CBCT and DOM were used as a tested methods.

4.2. Sample size determination:

Based on previous study of Monika et al. [(105)], a sample size of 157 teeth results in a two-sided 95% confidence interval with a width of 0.250 if the value of κ is 0.798 and the standard deviation SD(κ), is 0.798 for comparison between the three methods " done by: G*Power program (University of Düsseldorf, Düsseldorf, Germany)" [(106)].

4.3. Selection and preparation of the teeth.

Out of 200 freshly extracted mandibular first molars, 160 molars were collected from the outpatient clinic of the oral surgery department at the Faculty of Dental Medicine Al-Azhar University. The collected teeth were examined radiographically in mesio-distal & bucco-lingual directions and examined externally using DOM at 16X magnification. The mandibular first molar samples were selected according to specific inclusion criteria as follow;

- Teeth have fully formed apices.
- Teeth have two roots only.
- Teeth that were extracted due to periodontal problems, for prosthetic reasons, or carious teeth in those patients that rejected root canal treatment.

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While any tooth did not match the inclusion criteria of the study was excluded such as;

- Previously endodontic treated teeth.
- Teeth showing internal or external root resorption.
- Teeth showing pulp calcification and/or stones.
- Teeth have immature apecies.
- Teeth have any type of root fracture.
- Teeth have rather than two roots.

One hundred sixty mandibular first molars were used in this study while 40 mandibular first molars were excluded. The selected samples were washed immediately after extraction and placed in 10% formalin for 7 days. The samples were cleaned from any hard deposits using an ultrasonic scaler (Guilin Woodpecker Medical Instrument Company, Guangxi, China) and they were immersed in 2.5% sodium hypochlorite (NaOCl) (Egyptian Company for House-Hold Bleach, Cairo, Egypt) for 30 minutes to remove adherent soft tissue. Any soft tissue debris remained on the root surfaces were scraped using a scalpel blade. The selected samples were stored in normal saline solution (Egypt Otsuka Pharmaceutical Company, 10th of Ramadan City, Egypt) at room temperature till the time of use.

4.4. Construction of the plastic molds.

Sixteen circular plastic molds were constructed (90 mm in diameter and 15 mm in thickness) containing 10 rounded holes (15 mm in diameter and 15 mm in height) modified from Alkhawas M. 2011[107]. Two intersecting lines were drawn on the occlusal side of the mold for easy identification of the buccal, lingual, mesial, and distal surfaces. Further identification of the mesial surface of the samples was done using box shape amalgam dot at the occlusal side of the mold. (Figure no. 1)

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4.5. Holding of the samples inside the plastic molds.

The roots of each sample were painted with two successive layers of colored nail polish (Yolo, Yolo Cosmetics, Cairo, Egypt). Following placement of the mold on a glass slab (10 X 10 cm), each hole was filled with softened pink wax (El-Kods Wax Company, El-Mansoura, Egypt). Each sample was embedded in the softened wax till the level of cementoenamel junction (CEJ) while maintaining each surface of the sample in the same position of the corresponding surfaces on the mold (buccal, lingual, mesial, and distal) (Figure no. 2).

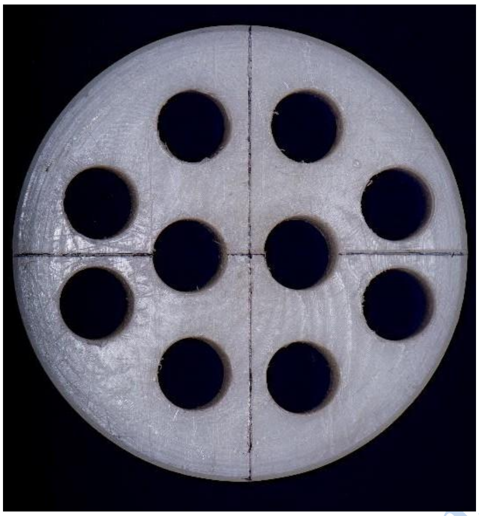

Figure (1): the plastic mold used in study (modified from Alkhawas M.)

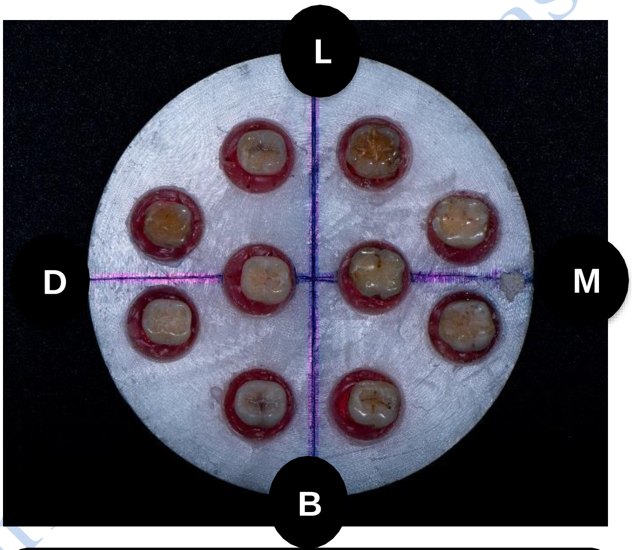

Figure (2): the plastic mold after the teeth were fixed on its hole in pink wax.

4.6. Methods of exploration of middle mesial (MMC) and middle distal (MDC) canals.

For calibration purposes of the endodontic researcher, an interrater reliability test was performed for each exploration method. Based on the interrater reliability test, 35 samples were randomly selected to be explored for the presence or absence of MMC&MDC by the three observers (two experienced endodontists with not less than a 3-year post-doctoral experience and one endodontic researcher) separately. The researcher collected the results in an excel sheet from all observers to be statistically analyzed in the form of a percentage of agreement among all observers using the Cohen kappa test. As the kappa value was superior to 0.798 (substantial agreement), the endodontic researcher continued the remaining 125 samples. Exploring the prevalence (presence or absence) of both MMC&MDC was done using three different methods high-resolution CBCT scanning of the teeth, dental operating microscope (DOM) at 16 x magnifications, and clearing/staining technique.

4.6.1. Exploration of the samples using high-resolution "CBCT" (CBCT exploration method)(figure: 3 and 4).

Scanning the samples was done using Planmeca ProMax 3D mid machine (Planmeca Pro Max 3D Mid Machine, planmeca Company, Helsinki, Finland) at 90 kV, 12 mA, a voxel size of 150 µm, and 15 seconds exposure time.

The scanned samples were concomitantly examined in axial, coronal, and sagittal views using Planmeca Romexis Viewer software (version 5.2). The values of image contrast and brightness were constantly adjusted using the software image-processing tool to ensure optimal standardization. (figure: 3 and 4)

4.6.2. Exploration of the samples using the dental operating microscope (DOM exploration method) (figure: 5 and 6).

Access cavity preparation and negotiation of the root canal orifices were done under16 X magnification using DOM (OMS2350 Dental Microscope, Zumax Medical Company, Jiangsa, China).

Initial penetration and deroofing of the samples were done using No. 2 round bur (SS White Burs, Inc, New Jersy, USA) mounted on high speed handpiece (NSk, Tochigi, Japan) with coolant. Initial exploration of the root canal orifices was done using No.DG16 endodontic explorer (DG16, Kerr Company, USA) Finishing of the access cavity walls was done using Endo-Z bur (Dentsply Maillefer, Ballaigue, Switzerland). Concomitantly irrigation of the pulp chamber was done using 2 ml of 5.25 % Naocl irrigating solution. Wall refining of the pulp chamber was done using ultrasonic wall refining tip (E3D, Guilin Woodpecker Medical Instrument Company, Guangxi, China) followed by scouting of the root canal orifices using ultrasonic canal scouting tip (SB1, Guilin Woodpecker

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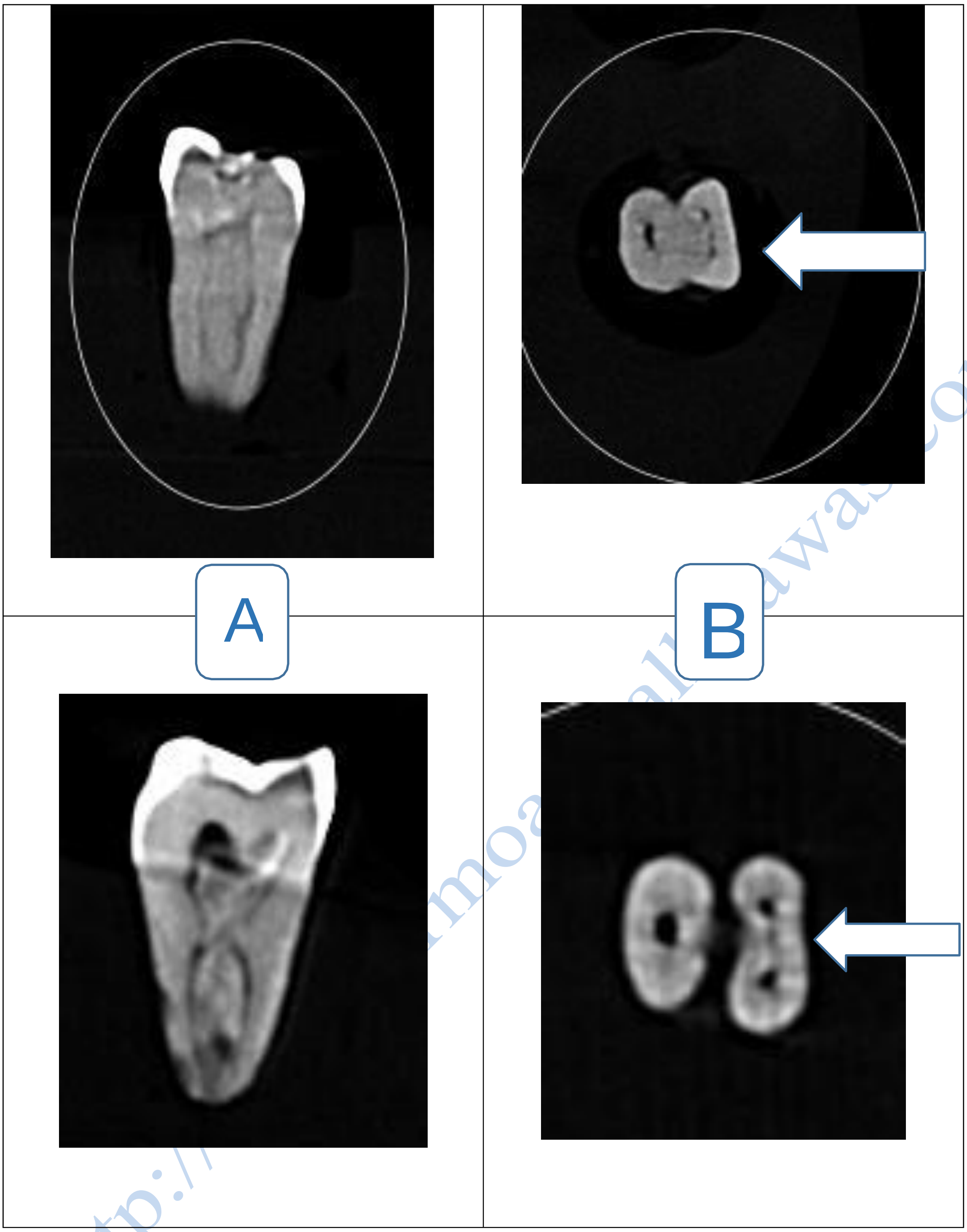

Figure (3): A CBCT scans showing prevalence of the middle mesial canal from coronal (A) and axial (B) views.

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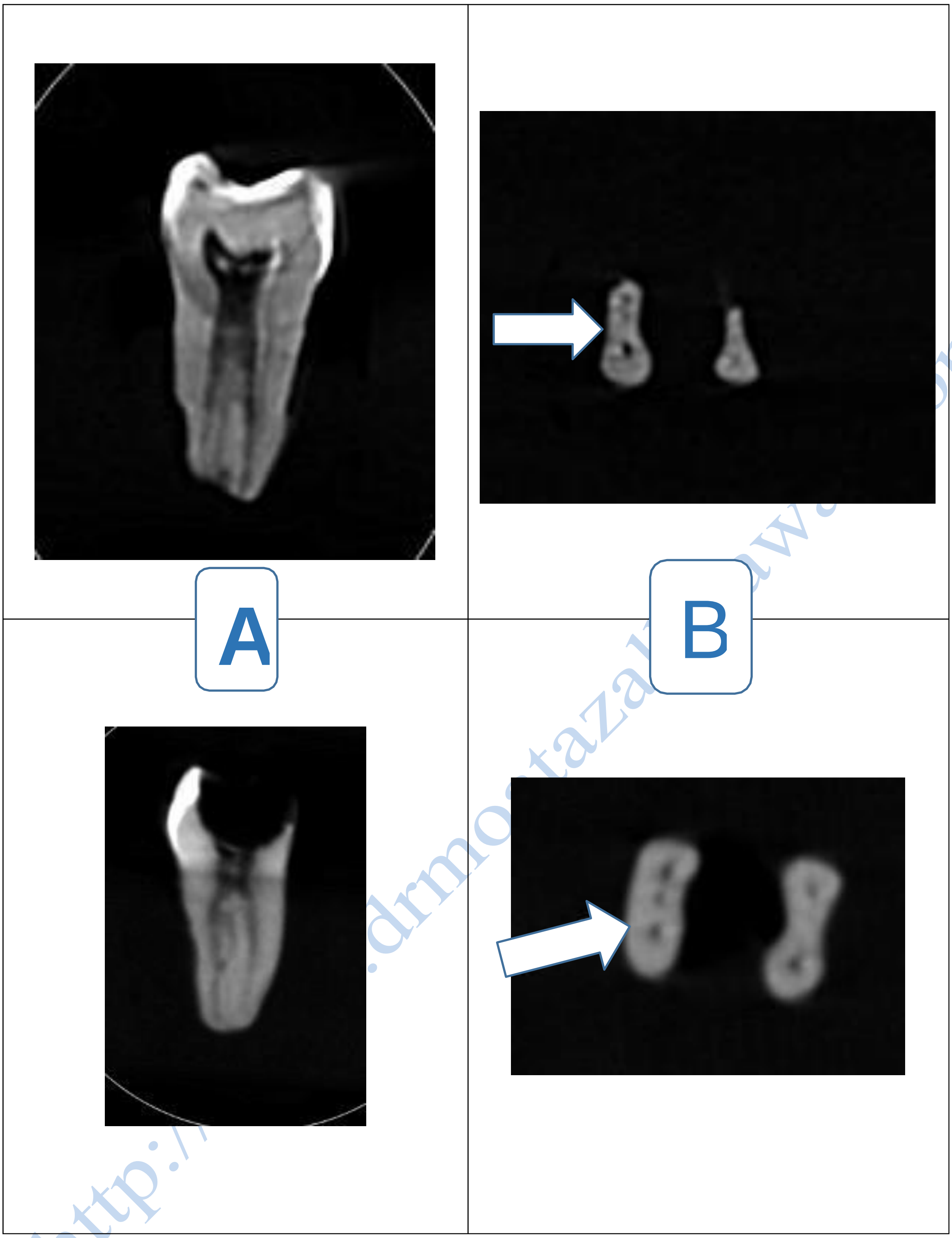

Figure (4): A CBCT scans showing prevalence of the middle distal canal from coronal (A) and axial (B) views.

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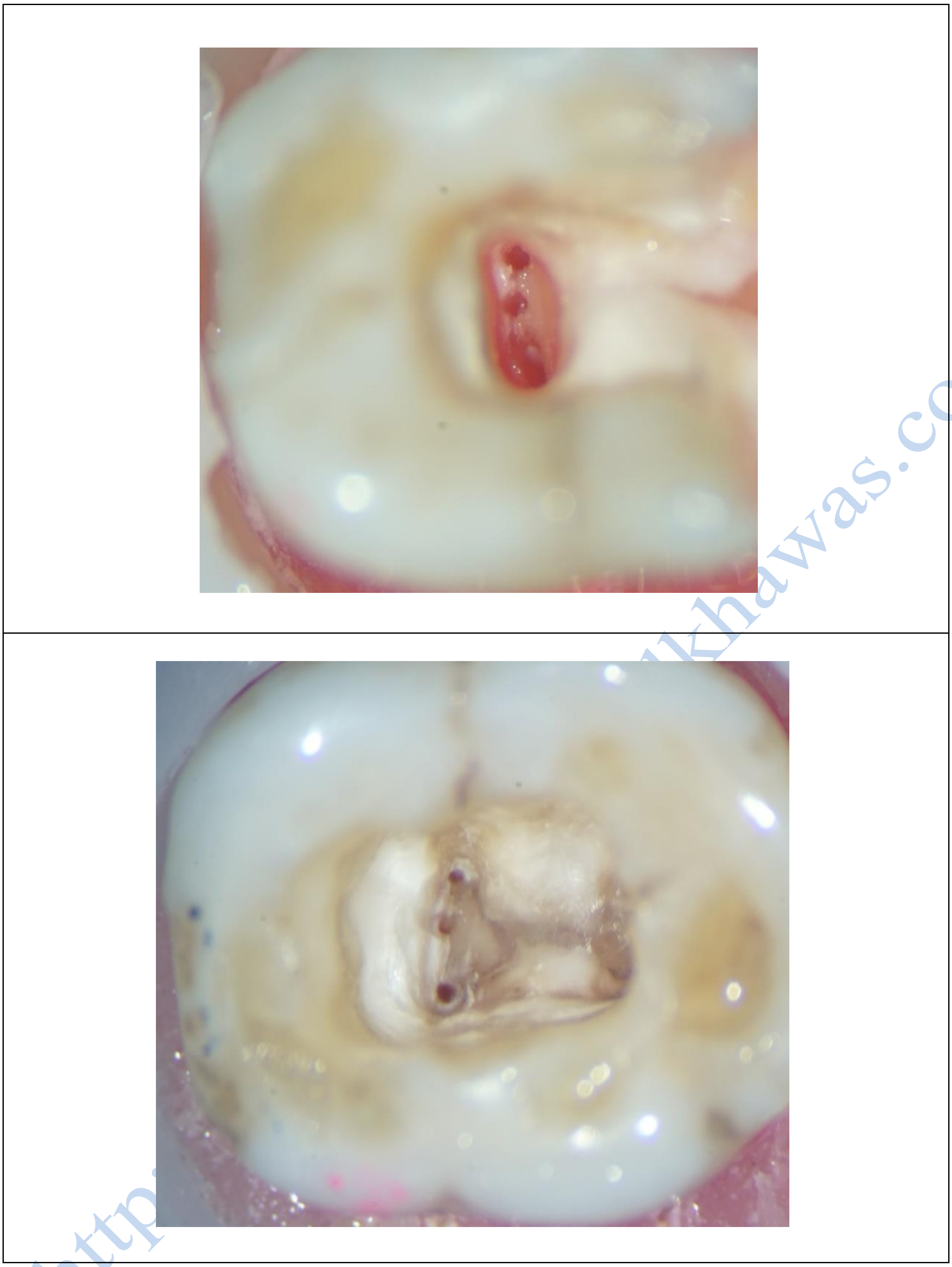

Figure (5): A photograph showing different teeth while exploring the middle mesial canal orifices.

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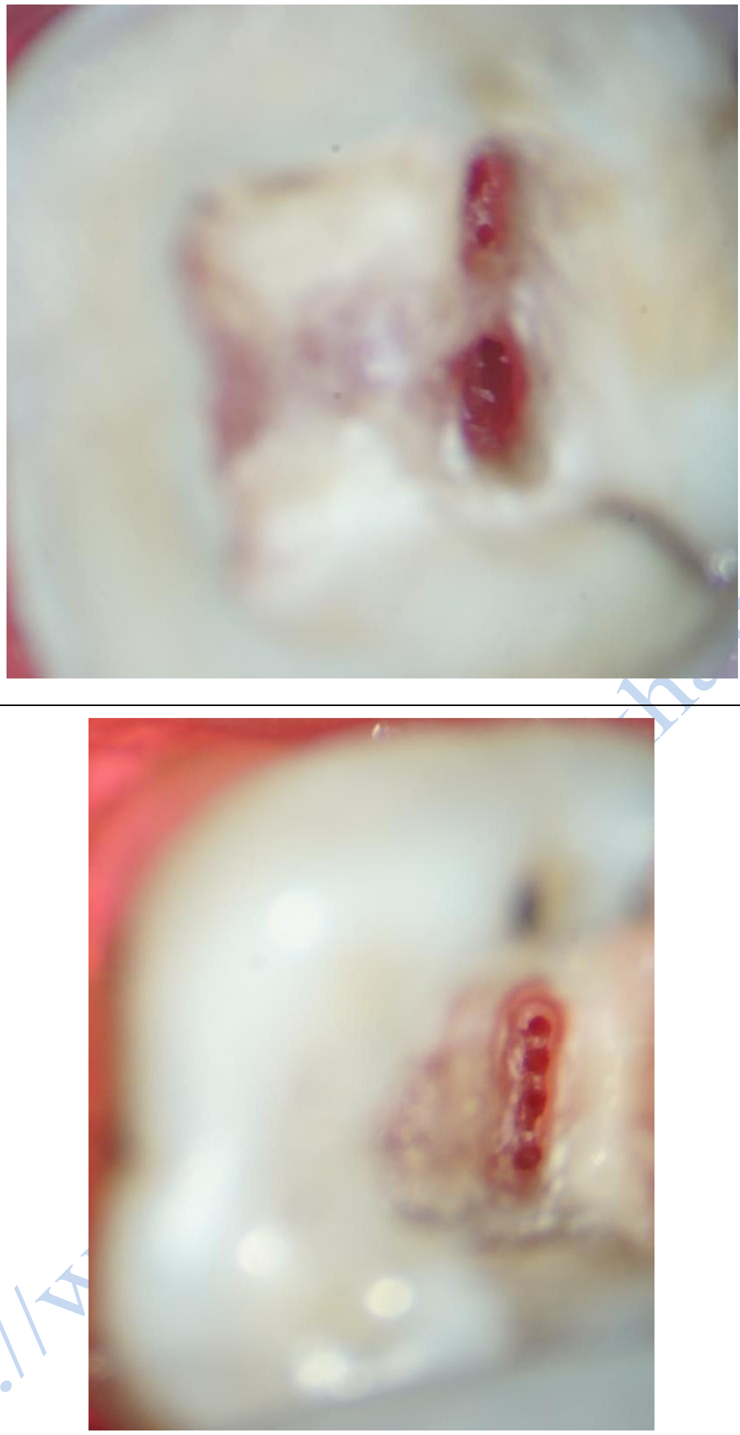

Figure (6): A photograph showing different teeth while exploring the middle distal canal orifices.

Medical Instrument Company, Guangxi, China). Irrigation of the pulp chamber using 2 ml of 5.25% Naocl irrigating solution followed by exploration and negotiation of the root canal orifices with K-file 0.08# (Dentsply Maillefer, Ballaigue, Switzerland) were done. (Figure: 5, 6)

4.6.3 . Exploration of the cleared samples using the dental operating microscope (clearing/staining exploration method). (figure: 7,8 and 9)

The samples used in the study were cleared, stained and examined using the following steps.

- Clearing of the samples.

The samples were removed from mold and the colored nail polish was scraped by scalpel to completely remove it from all root surfaces. Each sample placed in 5.25% sodium hypochlorite for 24 hours to dissolve organic debris and remnants of pulp and washed in tap water. The samples were decalcified in 5% nitric acid (NA255, Alpha Chemika Company, Mumbai, India) at room temperature. The nitric acid was changed daily for four days and samples in the container were agitated several times each day. After decalcification, washing the samples in tap water and submitted to dehydration under ascending concentrations of ethyl alcohol(2019/1, El Nasr Pharmaceutical Chemical Company, Cairo, Egypt) starting from 70% ethyl alcohol solution for one day, followed by 95% solution for one day and 99% ethyl alcohol for 3 days. The dehydrated samples were placed in methyl salicylate (2018/2, El Nasr Pharmaceutical Chemical Company, Cairo, Egypt) for 12 hours to make the dehydrated samples transparent. (Figure: 7)

- Staining of the samples.

The cleared samples were stained by injection of India ink dye (Rotring Inc, Hamburg, Germany) into the pulp chamber using a 27-gauge flat end needle. The dye was allowed to flow through the canal system by generating a negative

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pressure on the apices of the tooth through vacuum suction. The superfluous dye was removed from the teeth using gauze soaking with 99% concentration of ethyl alcohol. (Figure: 7)

- Examination of the cleared/stained samples.

Finally, the cleared samples were examined under DOM at 16X magnification to detect the presence or absence of MMC and MDCs. (Figure: 19, 9)

4.7. Statistical analysis

Data were represented by the total number, the percentage for each method used in the study. Descriptive statistics, Pearson correlation (r) test was used for comparing the relationship between different groups. Statistical analysis was performed with IBM®* SPSS®Θ Statistics Version 20.

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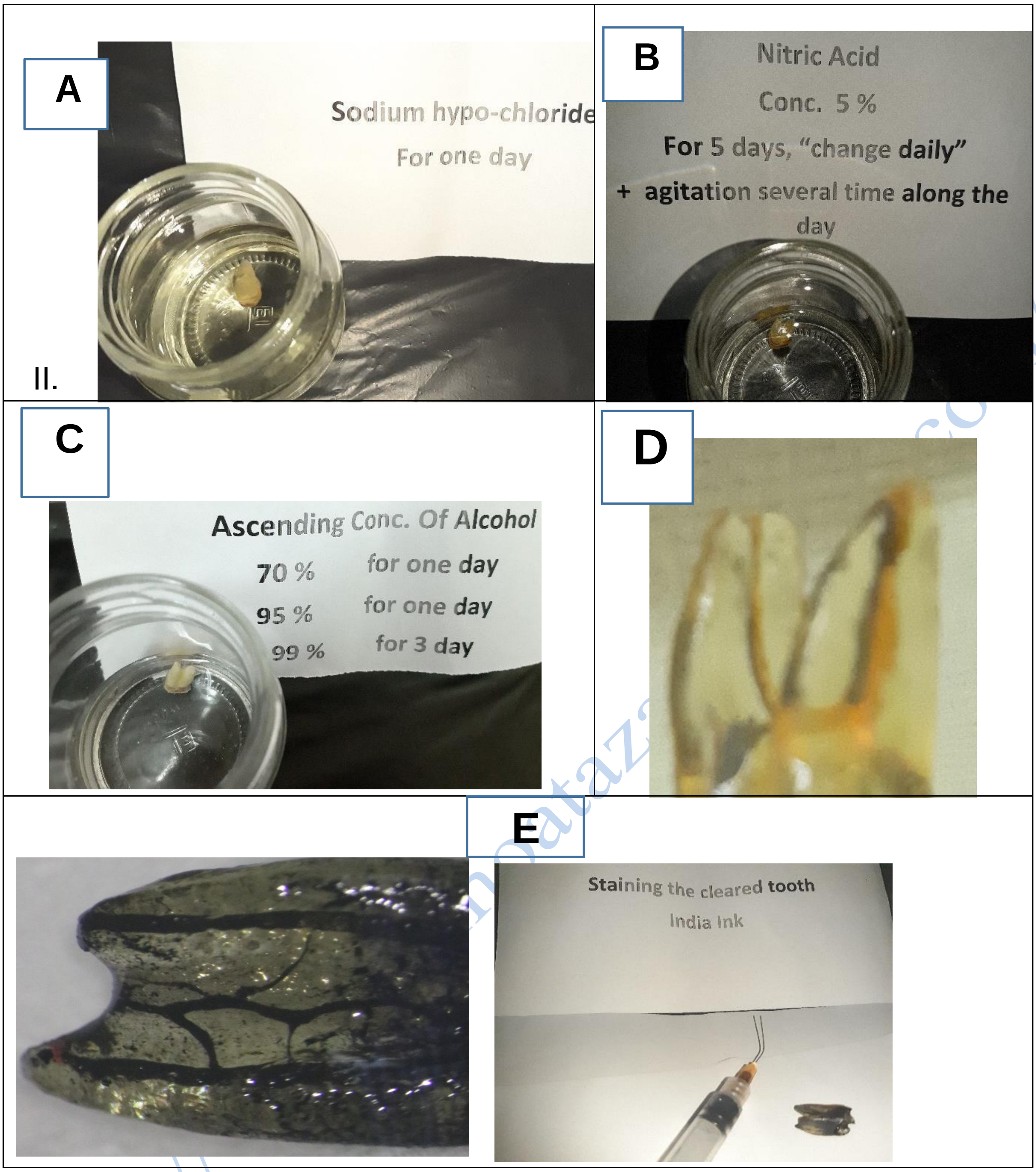

Figure (7): A photograph showing steps of clearing/staining, (A) tooth in sodium hypochlorite for one day, (B) tooth in nitric acid "for 4 days and changed daily", (C) tooth in ascending concentration of alcohol "70%, 95%, 99% for one day, one day, and 3 days", (D) tooth become transparent after submerge it in methyl salicylate for 12 hour and (E) cleared tooth after staining.

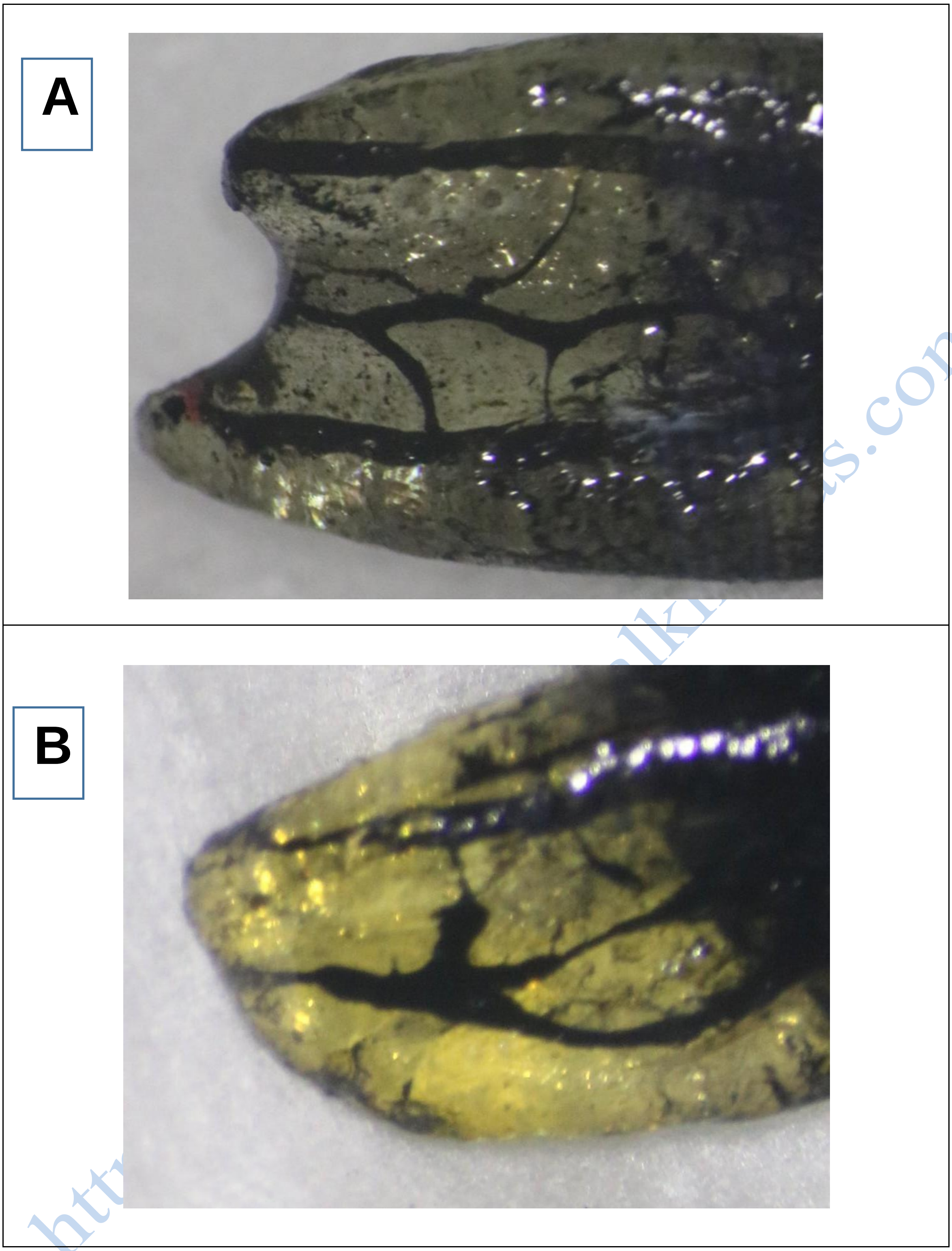

Figure (8): A photograph showing different cleared/stained teeth while exploring the middle mesial canal (A,B)

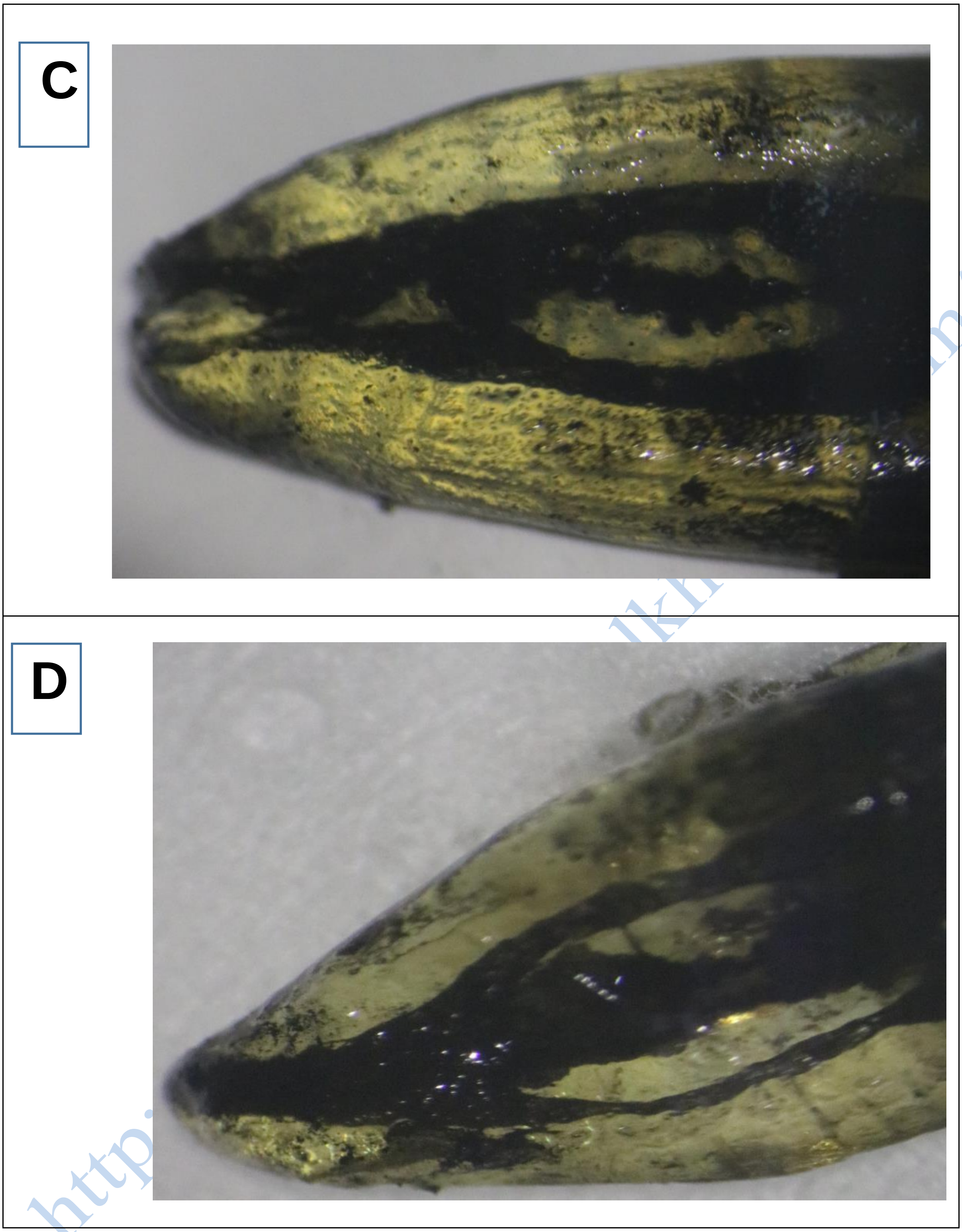

Figure (8): A photograph showing different cleared/stained teeth while exploring the middle mesial canal (C, D)

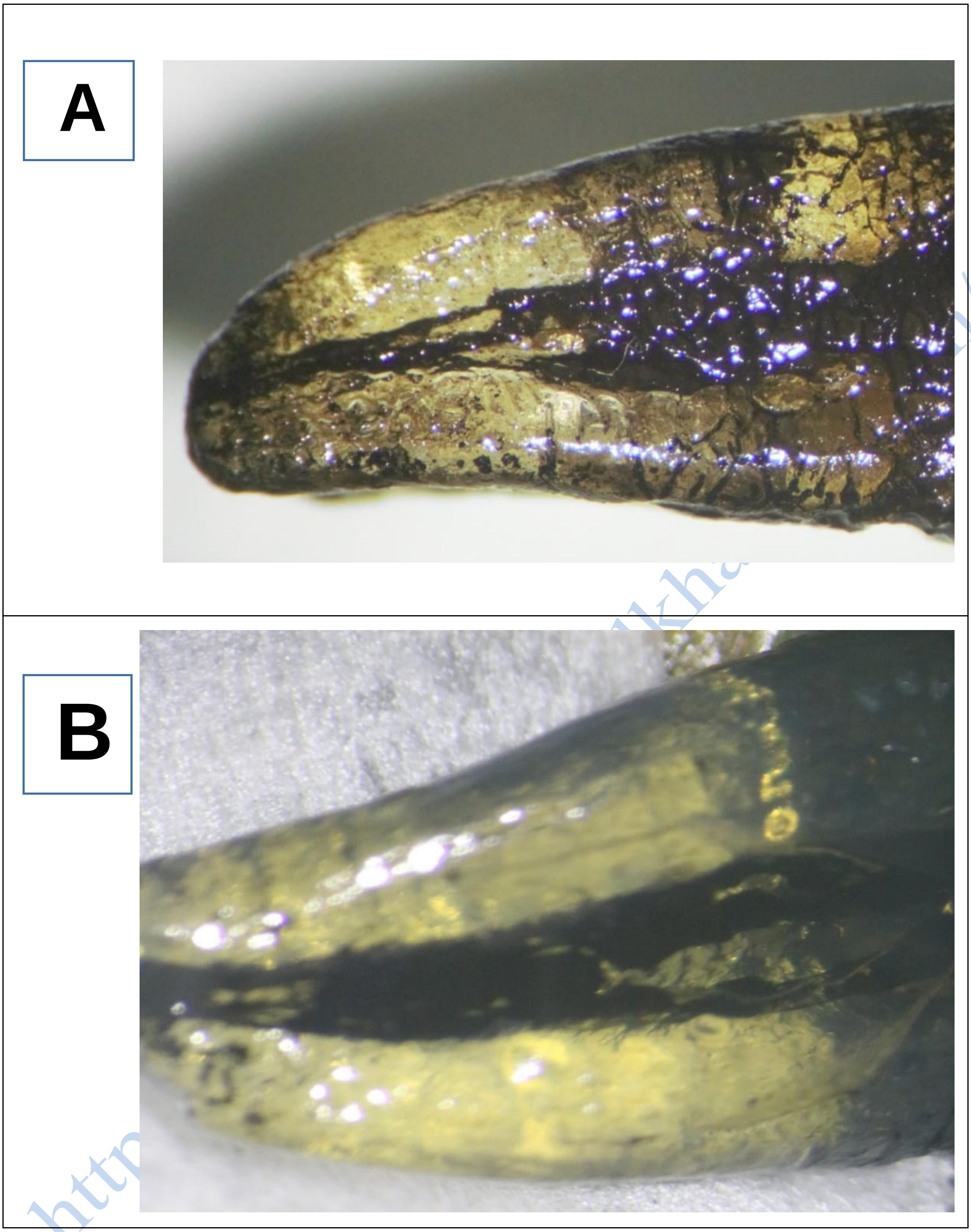

Figure (9): A photograph showing different cleared/stained teeth while exploring the middle distal canal (A, B)

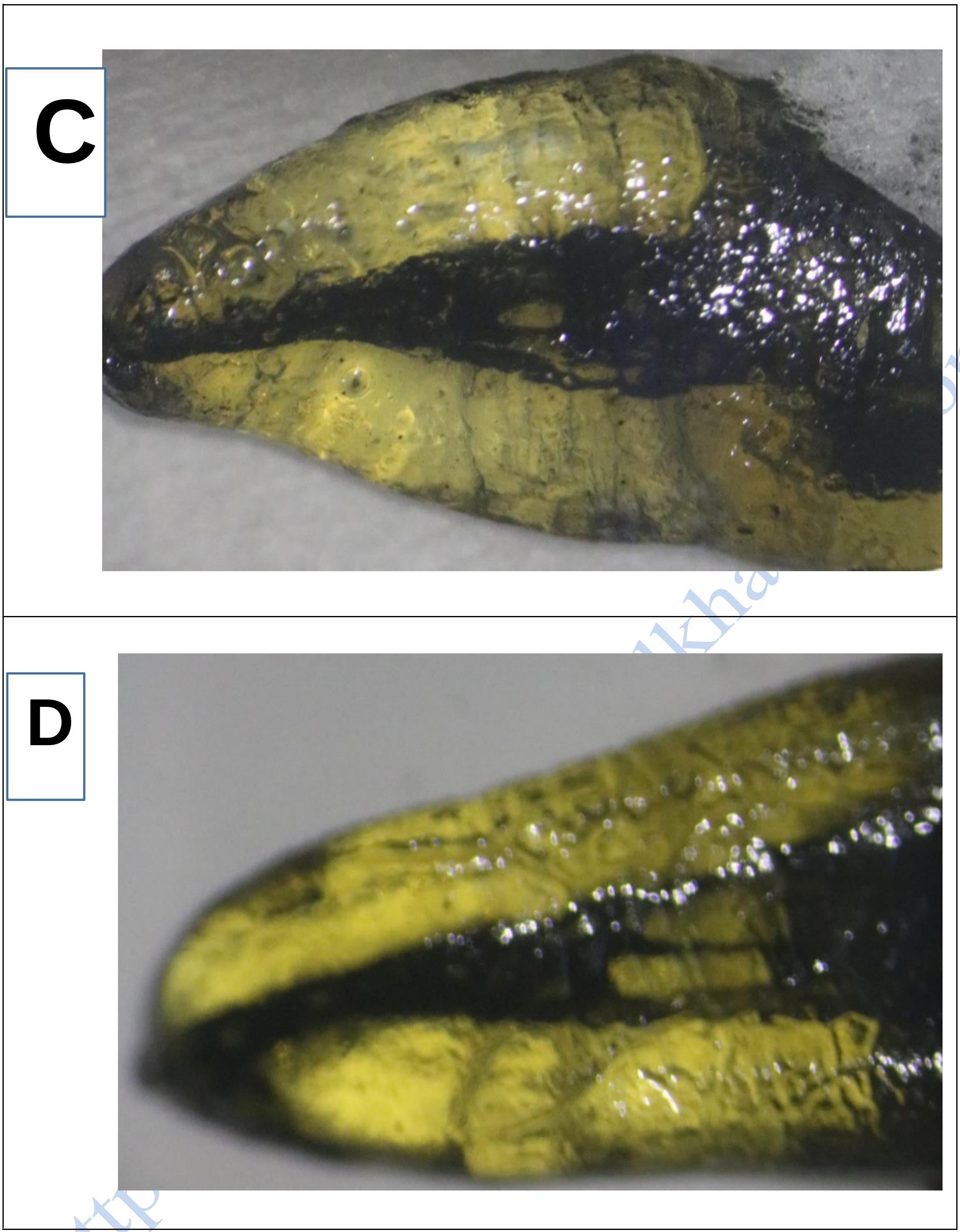

Figure (9): A photograph showing different cleared/stained teeth while exploring the middle distal canal (C, D)

III. In-vivo study:

4.8. Study design:

The in-vivo section of the study was designed as a cross-sectional, descriptive, observational study in the form of the prevalence of the middle mesial (MM) and middle distal (MD) canals.

4.9. Sample size determination:

The power analysis for the assessment of the prevalence of middle mesial and middle distal canals in mandibular first molars in an Egyptian population using the CBCT; based on data from previous study (Martins et al. [108], 2018) explored a total number of 1060 samples that have to be examined in the study [109].

4.10. Selection of the CBCT scans of the individuals:

Out of 1250, 1060 mandibular first molar were selected from 1150 CBCT scans of an Egyptian population which were collected from preexisting CBCT databases of a private dentomaxillofacial radiology center for those individuals that exposed to radiation for purpose rather than the present study in period between April 2016 to August 2019. Each CBCT scan had at least one mandibular first molar. The mandibular first molar tooth samples were selected according to specific inclusion criteria as follows;

- The age of individuals ranged from 12-60 years.
- Teeth have fully formed apices.
- Teeth without any type of restoration.
- No previous endodontic treatment.
- Absence of large periapical lesions.
- Absence of root resorption.

While any tooth did not match the inclusion criteria of the study was excluded such as:

- Previously endodontic treated teeth.

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- Teeth showing internal or external root resorption.
- Teeth showing pulp calcification and/or stones.
- Teeth have immature apecies.
- Teeth have any type of root fracture or cracks.

4.11. CBCT analysis: (Figure: 10, 11)

For calibration purposes of the endodontic researcher, an interrater reliability test was performed. Based on the interrater reliability test, 220 tooth samples were randomly selected to be analyzed for the presence or absence of MMC&MDC by the three observers (two experienced endodontists with not less than 3-year post-doctoral experience and one endodontic researcher) separately. The researcher collected the results in an excel sheet from all observers to be statistically analyzed using in the form of a percentage of agreement among all observers using the Cohen kappa test. As the kappa value was superior to 0.798 (substantial agreement), the endodontic researcher continued the remaining 840 samples for exploring the prevalence of both MMC&MDC mandibular first molar.

Scanning the samples was done using the Planmeca ProMax 3D mid machine at 90 kV, 12 mA, a voxel size of 200 – 75 µm, and 12-15 seconds exposure time.

The CBCT images were analyzed for the prevalence of both MMC&MDC in the mandibular first molars using Planmeca Romexis Viewer software (5.2). Axial, coronal, and sagittal two-dimensional sections were displayed on the monitor and the complete data set was assessed. Fixing the values of image contrast and brightness was adjusted using the software image processing tool to ensure optimal standardization. Excel sheet was included sex, age, side, number of roots, number of root canals in each root and presence or absence of both MMC&MDC. (Figure: 10, 11)

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4.12. Statistical analysis:

Data were represented by the total number, the percentage for each method used in the study. Descriptive statistics, Pearson correlation (r) test was used for comparing the relationship between different groups. Statistical analysis was performed with IBM®* SPSS®Θ Statistics Version 20.

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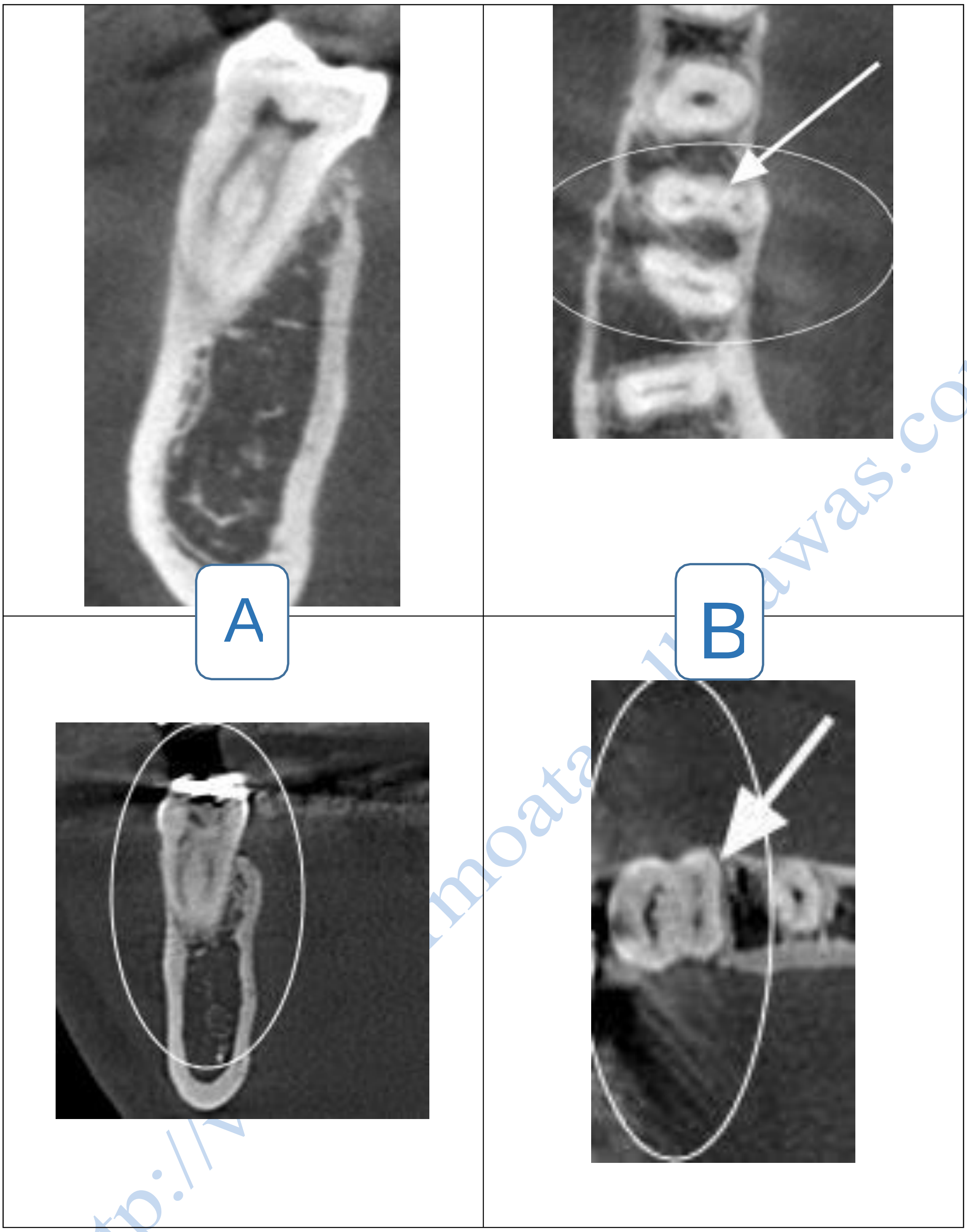

Figure (10): A CBCT scans showing prevalence of the middle mesial canal from coronal (A) and axial (B) views.

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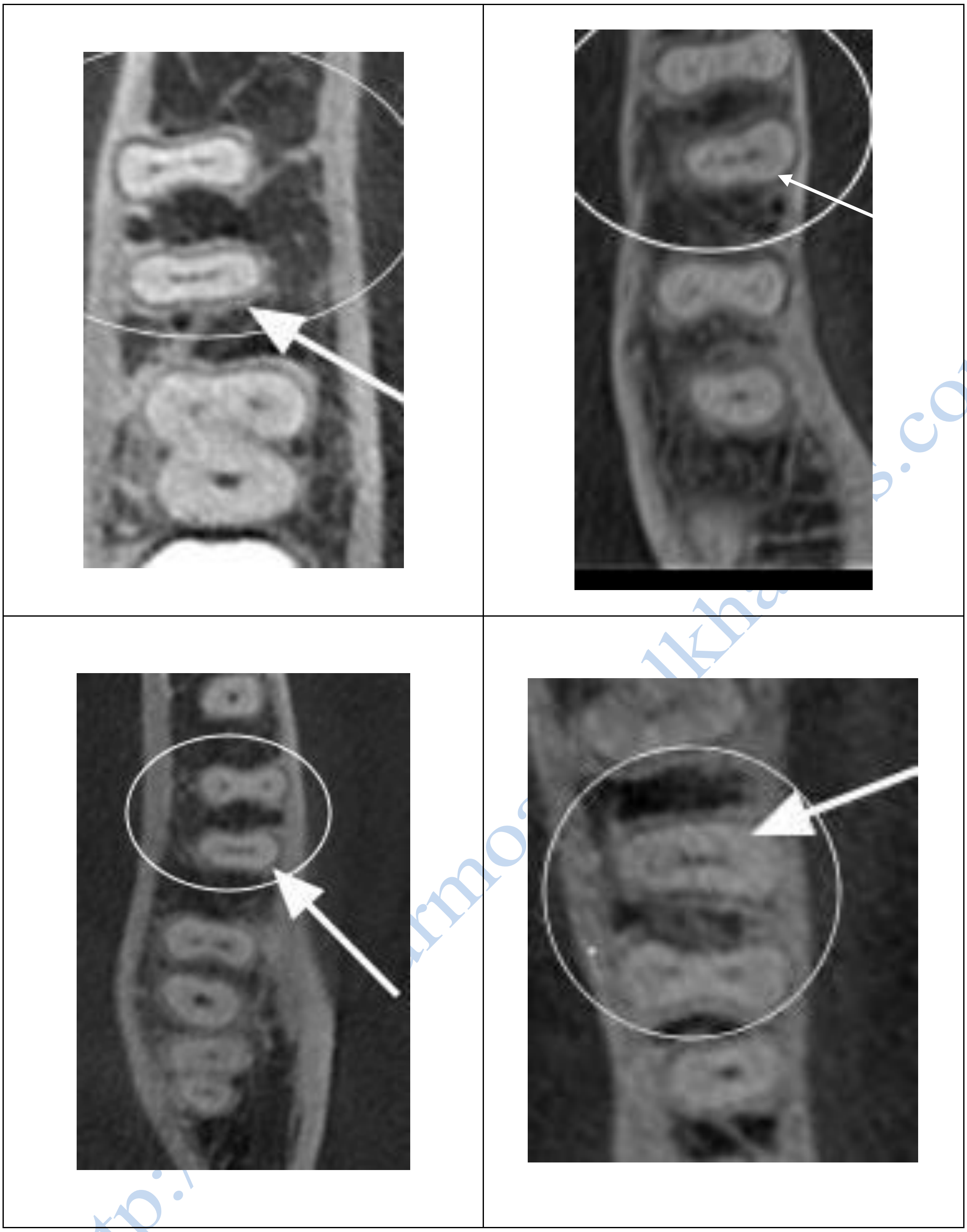

Figure (11): A CBCT scans showing prevalence of the middle distal canal from axial view.

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Results

I- Results of the In-vitro study.

5.1. Percentage of prevalence of the middle mesial and middle distal canals using each exploration method.

5.1.1. CBCT exploration method.

5.1.2. DOM exploration method.

5.1.3. clearing/staining exploration method.

5.2. Comparison of the percentage of prevalence of the middle mesial and the middle distal canals using different methods.

5.3. Correlative evaluation among the tested methods.

5.3.1. Correlative evaluation among the tested methods used for exploration of the middle mesial canal.

5.3.2. Correlative evaluation among the tested methods used for exploration of the middle distal canal.

II- Results of the In-vivo study.

5.4. Prevalence of the middle mesial and the middle distal canals.

5.5. Comparison of the sex distribution of the prevalence of the middle mesial and the middle distal canals.

5.6. Correlation between age of the patients and the prevalence of the middle mesial and the middle distal canals.

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Results

I- Results of the In-vitro study.

5.1. Percentage of prevalence of the middle mesial and middle distal canals using each exploration method. (Descriptive statistics)

5.1.1. CBCT exploration method (table no.1 &figure 12):

Among 160 (100%) samples, MMC was present in 35 (21.88%) samples and absent in 125 (78.13%).

MDC was present in 11(6.88%) samples and absent in 149 (93.13%).

Table no. (1): Percentage of prevalence of the middle mesial canals (MMC) and middle distal canals (MDC) using CBCT exploration method

No. of sample / Canals	Total		Present		Absent	
	No. of sample	%	No. of sample	%	No. of sample	%
MMC	160	100 %	35	21.88 %	125	78.13%
MDC	160	100 %	11	6.88 %	149	93.13 %

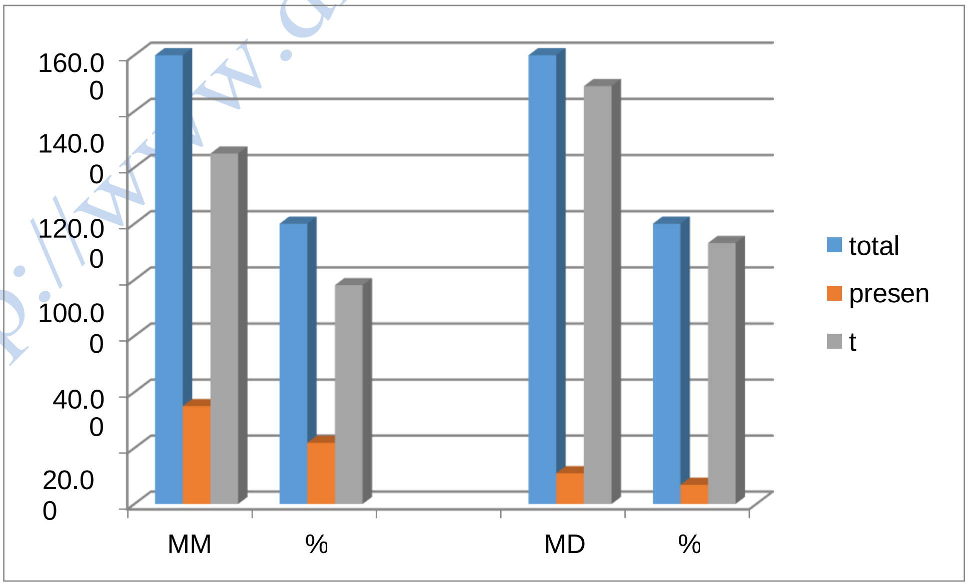

Figure (12): Percentage of prevalence of the middle mesial canals (MMC) and middle distal canals (MDC) using CBCT exploration method

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5.1.2. DOM exploration method (table no.2 &figure 13):

Among 160 (100%) samples, MMC was present in 46 (28.75%) samples and absent in 114 (71.25%).

MDC was present in 13(8.125%) samples and absent in 147 (91.875%).

Table no. (2): Percentage of prevalence of the middle mesial canals (MMC) and middle distal canals (MDC) using DOM exploration method

No. of sample / Canals	Total		Present		Absent	
	No. of sample	%	No. of sample	%	No. of sample	%
MMC	**160**	**100 %**	**46**	**28.75 %**	**114**	**71.25 %**
MDC	**160**	**100 %**	**13**	**8.125 %**	**147**	**91.875 %**

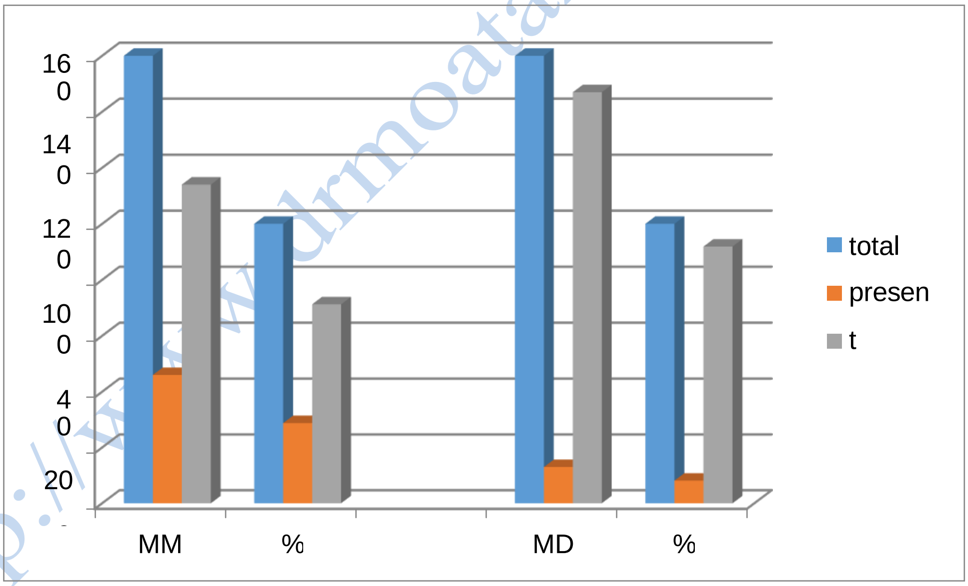

Figure (13): Percentage of prevalence of the middle mesial canals (MMC) and middle distal canals (MDC) using CBCT exploration method

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5.1.3. Clearing/staining exploration method (Table no.3 &figure 14):

Among 160 (100%) samples, MMC was present in 54 (33.54%) samples and absent in 106 (66.46%).

MDC was present in 12(7.45%) samples and absent in 148 (92.55%).

Table no. (3): Percentage of prevalence of the middle mesial canals (MMC) and middle distal canals (MDC) using clearing/staining exploration method:

No. of sample / Canals	Total		Present		Absent	
	No. of sample	%	No. of sample	%	No. of sample	%
MMC	160	100 %	54	33.54 %	106	66.46 %
MDC	160	100 %	12	7.45 %	148	92.55 %

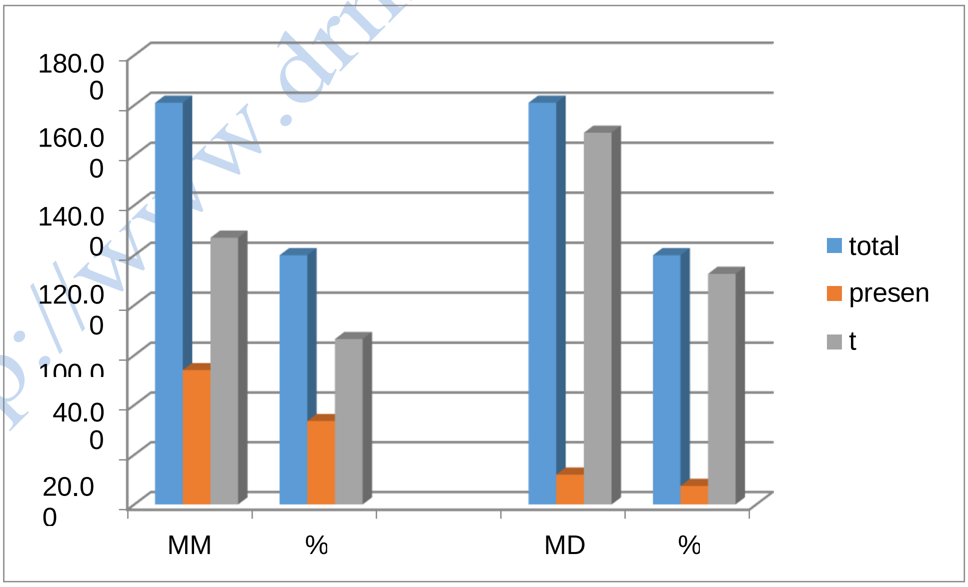

Figure (14): Percentage of prevalence of the middle mesial canals (MMC) and middle distal canals (MDC) using clearing/staining exploration method:

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5.2. Comparison of the percentage of prevalence of the middle mesial and the middle distal canals using different methods (Table no.4, 5 & figure 15,16).

This comparison done using Anova test.

- **The middle mesial canal (MMC):**

Among the 160 samples, clearing/staining exploration method showed the highest incidence of the middle mesial canal: 54 sample (33.54%) followed by the DOM exploration method: 46 sample (28.75%) then the CBCT exploration method: 35 sample (21.88%). (Table no. 4), (Figure 15)

There was no statistically significant difference between different methods CBCT exploration method, DOM exploration method, and clearing/staining exploration method. (p = 0.06)(Anova test) (Table no. 5), (Figure 16)

- **The middle distal canal (MDC):**

Among the 160 samples, the DOM exploration method showed the highest incidence of the middle distal canal: 13 samples (8.125%) followed by the clearing/staining exploration method: 12 samples (7.45%) then the CBCT exploration method: 11 samples (6.88%). (Table no. 4), (Figure 15)

There was no statistically significant difference between different methods CBCT exploration method, DOM exploration method and clearing/staining exploration method. (p = 0.8) (Anova test) (Table no. 5), (Figure 16)

Table no. (4): Comparison of the percentage of prevalence of the middle mesial and the middle distal canals using different exploration methods:

Exploration method. / Canals	CBCT exploration Method		DOM exploration Method		Clearing/staining exploration method	
	No. of sample	%	No. of sample	%	No. of sample	%
MMC	35	21.88 %	46	28.75 %	54	33.54 %
MDC	11	6.88 %	13	8.125 %	12`	7.45 %

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Table no. (5): The mean of percentage of prevalence of the middle mesial canal and the middle distal canal, (SD, P value) (Anova test):

` Exploration method. / Canals	CBCT exploration Method		DOM exploration method		Clearing/staining exploration method		P
	mean	SD(±)	mean	SD(±)	mean	SD(±)	
MMC	0.21	0.00	0.29	0.00	0.33	0.00	0.06
MDC	0.06	0.00	0.08	0.00	0.07	0.00	0.80

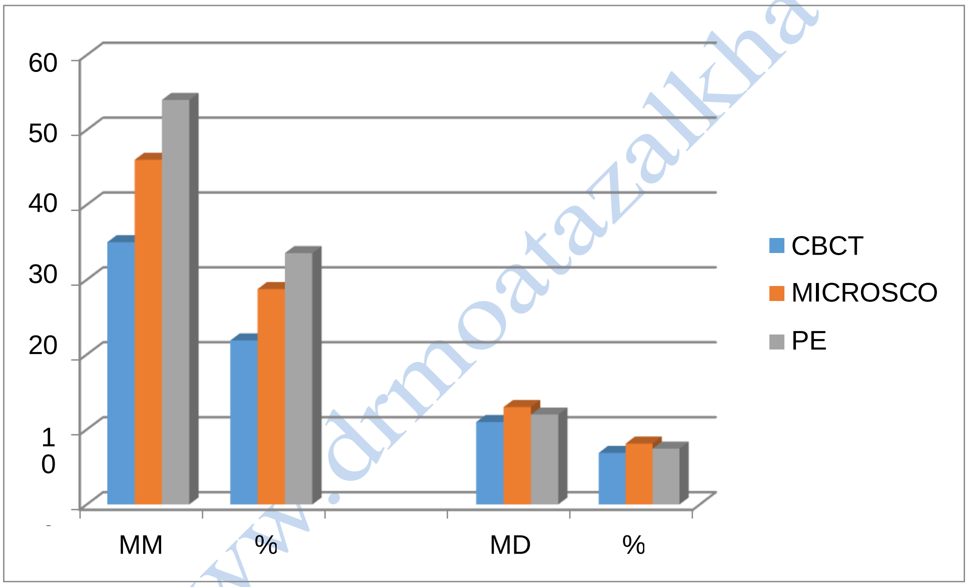

Figure (15): Comparison of the percentage of prevalence of the middle mesial and the middle distal canals using different exploration methods:

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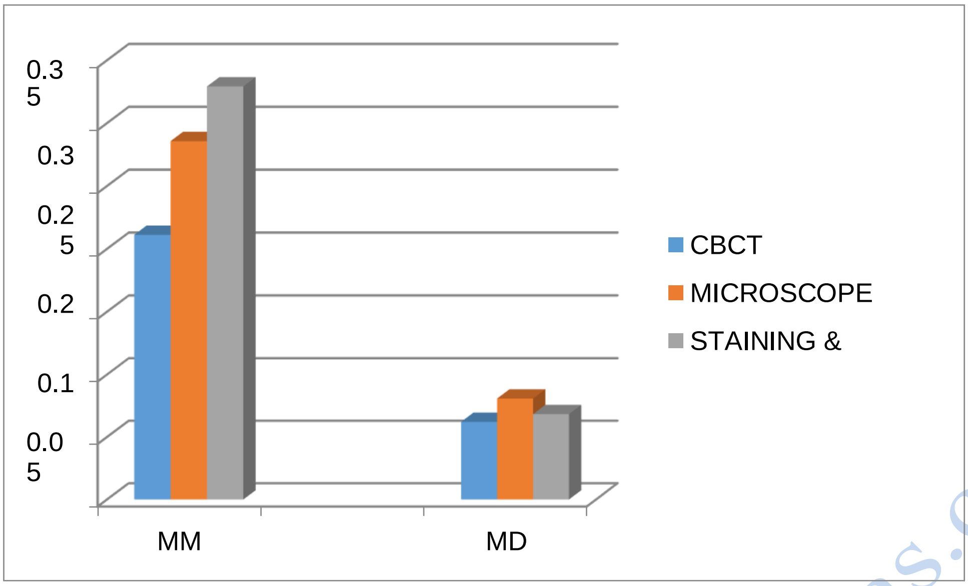

Figure (16): The mean of percentage of prevalence of the middle mesial canal and the middle distal canal(Anova test):

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5.3. Correlative evaluation among the tested methods.

The Correlative evaluation was done using Pearson correlation test through its degree of correlation ranging from zero to one as follow: (table no. 6)

Table no. (6): the degree of correlation of Pearson correlation test .

Pearson correlation (r) value	significance
1	Linear positive relation
1 - 0.9	Very strong positive relation
0.7 - 0.9	Strong positive relation
0.5 - 0.7	Medium positive relation
0.3 - 0.5	Weak positive relation
0 - 0.3	Very weak or no relation

5.3.1. Correlative evaluation among the tested methods used for exploration of the middle mesial canal. (Table no.7 & figure 17)

There is a weak positive relation between the CBCT exploration method and the DOM exploration method as r = 0.38, means; the prevalence of the middle mesial canal in the CBCT exploration method increases its prevalence when DOM exploration method used.

There is a very weak positive relation between the CBCT exploration method and clearing/staining exploration method as r = 0.28, means; the prevalence of the middle mesial canal in the CBCT exploration method increases its prevalence when clearing/staining exploration method used in a little manner.

There is a positive relation between DOM exploration method and clearing/staining exploration method as r =0.7, means; the prevalence of the middle mesial canal in DOM exploration method increases its prevalence when clearing/staining exploration method used.

Table no. (7): Correlative evaluation among the tested methods used for exploration of the middle mesial canal.

exploration methods	CBCT exploration method	DOM exploration Method	Clearing/staining exploration method
CBCT exploration method	1.00		
DOM exploration method	0.38	1.00	
Clearing/staining exploration method	0.28	0.70	1.00

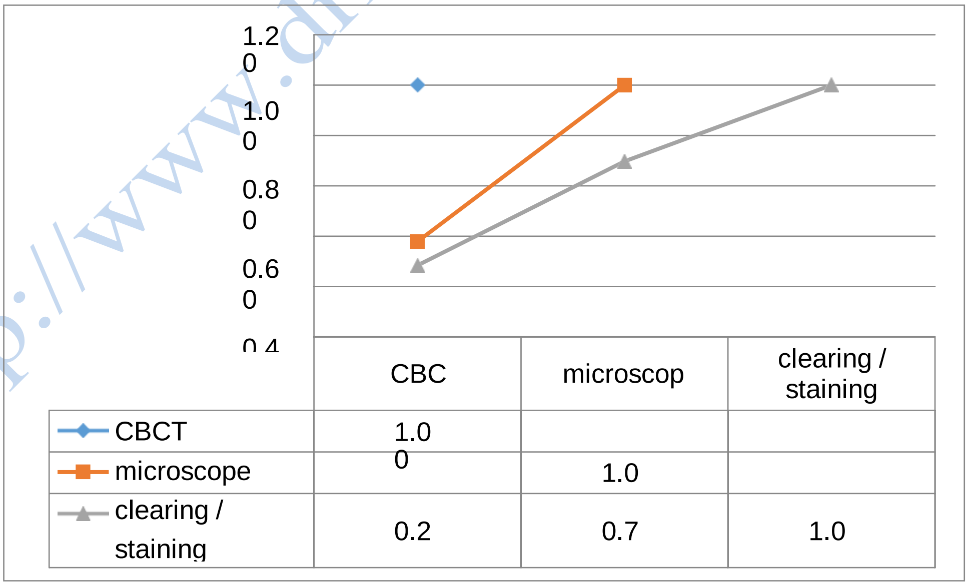

Figure (17): Correlative evaluation among the tested methods used for exploration of the middle mesial canal

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5.3.2. Correlative evaluation among the tested methods used for exploration of the middle distal canal. (Table no.8 & figure 18).

There is a very weak relation that seems that no relation between the CBCT exploration method and DOM exploration method as r =0.21

There is a weak positive relation between the CBCT exploration method and the clearing/staining exploration method as r =0.34, that means; the prevalence of the middle distal canal in the CBCT exploration method increases its prevalence when clearing/staining exploration method used.

There is a positive relation between the DOM exploration method and clearing/staining exploration method as r =0.55, that means; the prevalence of the middle distal canal in the DOM exploration method increases its prevalence when clearing/staining exploration method used.

Table no. (8): Correlative evaluation among the tested methods used for exploration of the middle distal canal.

exploration methods	CBCT exploration method	DOM exploration method	Clearing/staining exploration method
CBCT exploration method	1.00		
DOM exploration method	0.21	1.00	
Clearing/staining exploration method	0.34	0.55	1.00

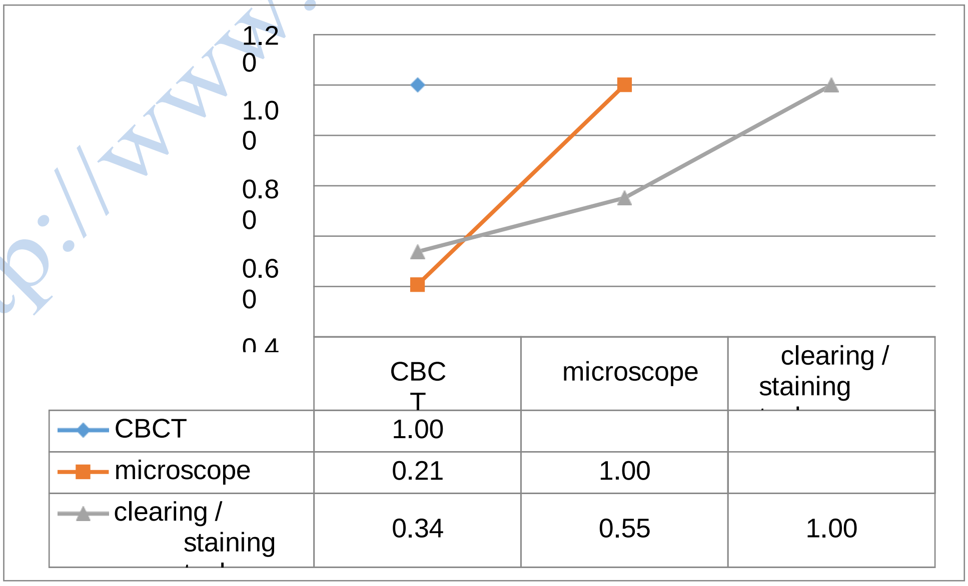

Figure (18): Correlative evaluation among the tested methods used for exploration of the distal mesial canal

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II- Results of the In-vivo study.

5.4. Prevalence of the middle mesial and the middle distal canals. (Table no.9 & figure no.19).

- **The middle mesial canal:**

Among 1060 (100%) sample, the total number of the middle mesial canals were found: 233 (21.98%), and the absence was 827 (78.02 %).

- **The middle distal canal:**

Among 1060 (100%) sample, the total number of the middle distal canals were found: 67 (6.32 %), and the absence was 993 (93.68 %).

Table no. (9): Prevalence of the middle mesial and the middle distal canals of the In-vivo study using CBCT exploration.

No. of sample / Canals	Total		Present		Absent	
	No. of sample	%	No. of sample	%	No. of sample	%
MMC	1060	100 %	233	21.98 %	827	78.02 %
MDC	1060	100 %	67	6.32 %	933	93.68 %

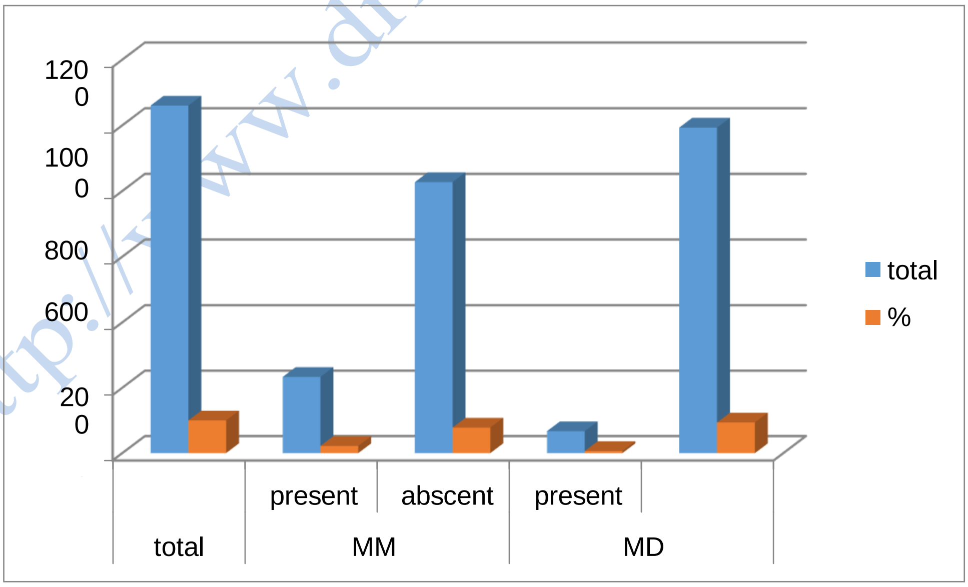

Figure (19): Prevalence of the middle mesial and the middle distal canals of the In-vivo study using CBCT exploration.

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5.5. Comparison of the sex distribution of the prevalence of the middle mesial and the middle distal canals. (Table no.10,11 & figure 20,21).

- The middle mesial canal:
  - Among 233 sample containing the canal, 100 sample (42.92%) found in males while 133 sample (57.08%) found in females. ***(Table no.10)(figure. 20)***
  - There is no any correlation between prevalence of the middle mesial canal and sex, as r =0.012. (***Pearson correlation test)(Table no.11)(figure 21)***

- The middle distal canal:
  - Among 67 sample containing the canal, 31 sample (46.27%) found in males while 36 sample (53.73%) found in females. ***(Table no.10)(figure 20)***
  - There is no any correlation between prevalence of the middle distal canal and sex, as r =0.06. (***Pearson correlation test) (Table no.11)(figure 21)***

Table no. (10): the sex distribution of the prevalence of the middle mesial and the middle distal canals.

Sex distribution / Canals	Total		Males		Females	
	No. of sample	%	No. of sample	%	No. of sample	%
MMC	233	100 %	100	42.92 %	133	57.08 %
MDC	67	100 %	31	46.27	36	53.73

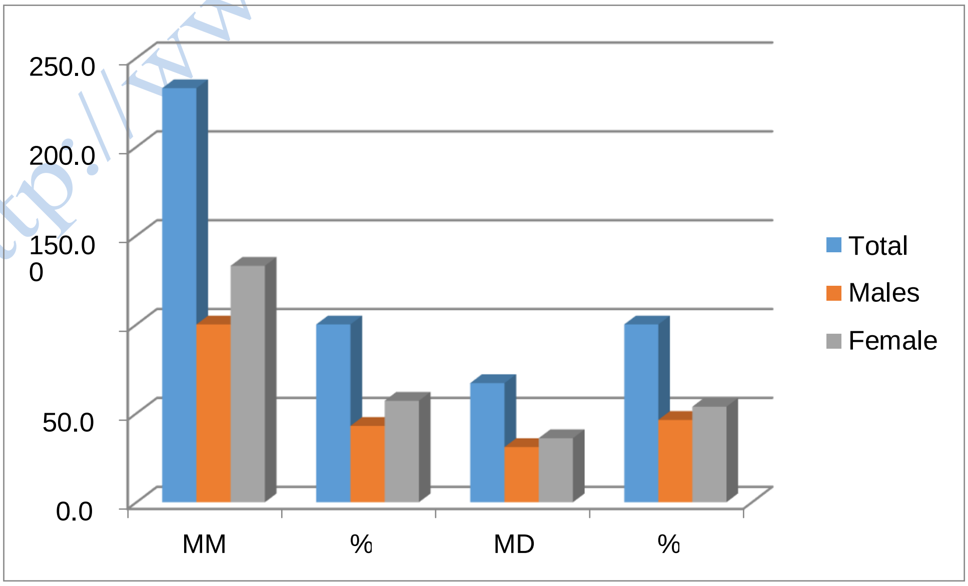

Figure (20): the sex distribution of the prevalence of the middle mesial and the middle distal canals.

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Table no.(11): Correlation between sex of the patients and prevalence of the middle mesial and the middle distal canals.((Pearson correlation test)

Canals	Sex (R)
MM	0.012
MD	0.06

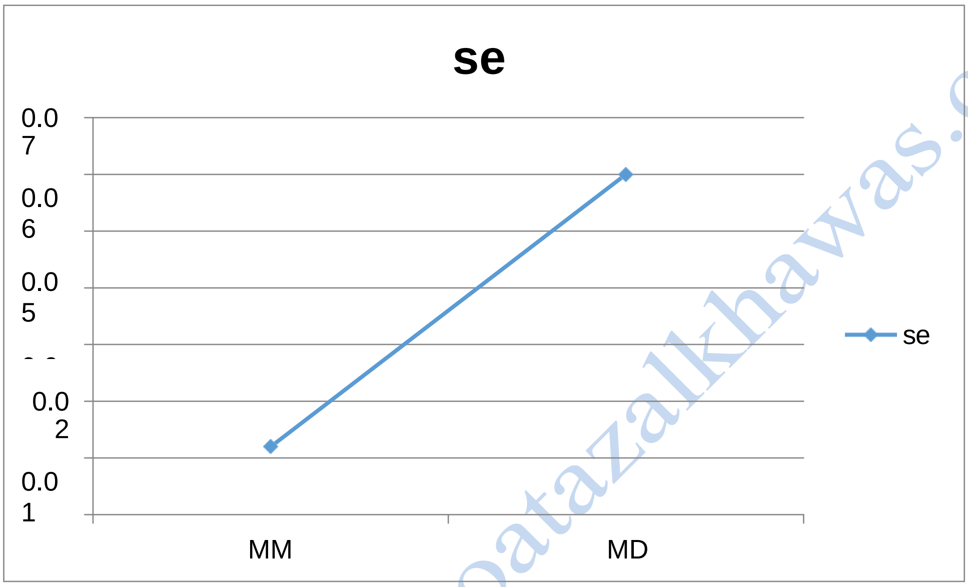

Figure (21): Correlation between sex of the patients and prevalence of the middle mesial and the middle distal canals. (Pearson correlation test).

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5.6. Correlation between age of the patients and the prevalence of the middle mesial and the middle distal canals. (Table no.12 & figure 22)

The Correlation was done using Pearson correlation test through its degree of correlation ranging from zero to one as shown in table no. (6)

The middle mesial canal:

There is no any correlation between prevalence of the middle mesial canal and age, as r =0.09

- **The middle mesial canal:**

There is no any correlation between prevalence of the middle distal canal and age, as r =0.15

Table no. (12): Correlation between age of the patients and prevalence of the middle mesial and the middle distal canals:

canals	Age(R)
MM	**0.09**
MD	**0.15**

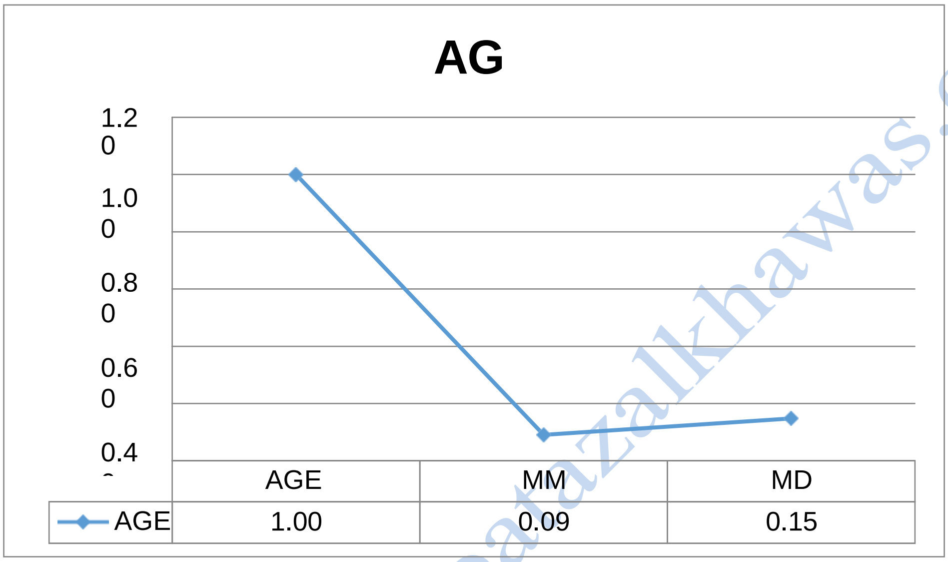

Figure (22): Correlation between age of the patients and prevalence of the middle mesial and the middle distal canals:

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Discussion

Thorough knowledge of canal morphology and its variations is mandatory for better treatment outcome as 48 % of the endodontic failure is due to missed anatomy [1,4,110].

A live example of the anatomical variations in canal morphology is the mandibular first molar that lies on the top of these variations as it is the first permanent tooth that erupts in the oral cavity and usually necessitate root canal treatment [7, 4].

Among the variations in canal morphology of the mandibular first molar is the prevalence of extra canals such as middle mesial and middle distal canals [7,28,29]. Different methods have been published in the literature to explore these variations in canal morphology ranging from historical to non-invasive ones [4, 111, 112].

The present study was done to evaluate the prevalence of the middle mesial and the middle distal canals in the mandibular first molar in the Egyptian population using three different methods for the In-vitro exploration, besides using CBCT for the In-vivo exploration.

The researcher received a condensed training on using CBCT and the dental operating microscope before starting the study followed by interrater exploration to ensure calibration of the researcher.

- In-vitro study:

Two hundred extracted first mandibular molars were collected, 40 mandibular first molars were excluded as shown in (table no.13), while 160 mandibular first molars were used in the study (Based on sample size determination). The roots of each sample were painted with nail polish to prevent blockage of the root openings with future embedding in pink wax that was done to secure the samples during CBCT scanning and access cavity preparation[107].

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Table (13): A table showing Different causes and the numbers of excluded teeth within an in-vitro study

Previously endodontic treated teeth	**8**
Teeth showing pulp calcification	**5**
Teeth have immature apecies	**3**
Teeth have any type of root fracture	**10**
Teeth have rather than two roots	**1**
Teeth were undergone to clearing/staining trials	**13**
Total	**40**

The plastic molds were constructed and modified from Alkhawas et al. 2011[107] to fix the samples inside by the aid of pink wax.

The CBCT was used as the first evaluation method due to its reliability in detecting the anatomical variations with different levels beside its availability [113]. While the dental operating microscope was used as the second evaluation method as it allows for better visibility, illumination while discovering more details for accurate detection of the root canal orifices[114, 115].

The clearing/staining exploration method was used as a positive control method due to it demonstrates three dimensional, morphological accurate, dyed pulpal spaces of a tooth that would be correctly positioned within a transparent tooth structure [116].

- **CBCT exploration method:**

Regarding the middle mesial canal (MMC), the results of this in-vitro study revealed that the prevalence of MMC is 21.88 % (35 of 160 total sample) in the tested samples (Voxel size: 150 micron). This result is convergent with the in-vitro results of Soares de Toubes et al.[113] on the Brazilian population that identified MMC in 27 % (12 of 44 total sample) of their tested samples using CBCT exploration method (Voxel size: 200 micron). While this result was in conflict with that in-vitro result of Mukhaimer et al. [117] on Palestinian population that identified MMC in 3.1 %(10 of 320 total sample) using CBCT exploration

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method (voxel size: unknown).This discrepancy in the prevalence of the MMC when explored with CBCT may be attributed to the race differences in addition to the difference in methodology including the sample size and the voxel size of each CBCT machine used.

Regarding the middle distal canal (MDC), the results of this in-vitro study revealed that the prevalence of MDC is 6.88 % (11 of 160 total sample) in the tested samples (Voxel size: 150 micron). Although upon searching, there was no available research found on the prevalence of MDC using CBCT exploration method, the present study is in harmony with the in-vitro results of Flipo-Perez et al.[29] on the Brazilian population that identified MDC in 8 % (8 of 100 total sample) (Voxel size: 19.6 micron).

- **DOM exploration method:**

Regarding the middle mesial canal (MMC), the results of this in-vitro study revealed that the prevalence of MMC is 28.75 % (46 of 160 total sample) in the tested samples (16 X magnification). This result in agreement with the in-vitro results of Soares de Toubes et al.[113] on the Brazilian population that identified MMC in 30 % (13 of 44 total sample) of their tested samples using DOM exploration method (13 X magnification). In addition, this result is convergent with the in-vitro results of Karapinar-Kazandag et al. [19] on Turkish population that identified MMC in 18.0% (9 of 48 Total sample) of their tested samples using DOM exploration method (30 X magnification). This discrepancy in the prevalence of the MMC when explored with DOM may be attributed to the race differences in addition to the difference in methodology including the sample size and the magnification power of each dental operating microscope used.

Regarding the middle distal canal (MDC), the results of this in-vitro study revealed that the prevalence of MDC is 8.125 % (13 of 160 total sample) in the tested samples (16 X magnification power). Upon searching, there was no available research found on the prevalence of MDC using DOM exploration method

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- **Clearing/staining exploration method:**

Regarding the middle mesial canal (MMC), the results of this in-vitro study revealed that the prevalence of MMC is 33.54 % (54 of 160 total sample) in the tested samples. This result is in conflict with that in-vitro result of Gulabivala et al. ([44] on Burmese population that identified MMC in 10.8% (15 of 139 total sample) using clearing/staining exploration method, and also in conflict with that in-vitro result of Al-Qudah et al [46] on Jordanian population that identified MMC in 6 %(10 of 330 total sample) using clearing/staining exploration method. This discrepancy in the prevalence of the MMC when explored with clearing/staining may be attributed to the race differences in addition to the difference in methodology including the sample size, nitric acid concentrations, alcohol dehydration and staining technique used.

Regarding the middle distal canal (MDC), the results of this in-vitro study revealed that the prevalence of MDC is 7.45 % (12 of 160 total sample) in the tested samples. This result is convergent with that in-vitro result of Ahmed et al. [118] on the Sudanese population that identified MDC in 3 % (3 of 100 total sample) using clearing/staining exploration method. This discrepancy in the prevalence of the MDC when explored with clearing/staining may be attributed to the race differences in addition to the difference in methodology in term of the clearing time with regarding to nitric acid concentrations and alcohol dehydration (concentration and duration) beside the staining technique used.

Regarding the different exploration methods used to detect the percentage of prevalence of the MMC and MDC, this in-vitro study revealed that the highest prevalence of the middle mesial canal in the selected samples was found when using the clearing/staining exploration method (33.54 %) followed by exploration using the DOM exploration method (28.75 %) while the least prevalence of the middle mesial canal was detected when using CBCT exploration method (21.88 %) with no statistical significant difference among

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the tested exploration method. In addition to, the study revealed that the highest prevalence of the middle distal canal in the selected samples was found when using the DOM exploration method (8.125 %) followed by exploration using the clearing/staining exploration method (7.45 %) while the least prevalence of the middle distal canal was detected when using CBCT exploration method (6.88 %) with no statistical significant difference among the tested exploration method. This results were in agreement with that in-vitro result of Monika et al.[(105)] that concluded although this result was not statistically significant among different methods (clearing/staining technique, DOM and CBCT) for analysis of root canal anatomy, yet overall these methods are more effective in negotiating MMC & MDC as compared to routine clinical techniques.

- In-vivo study:

A total of 1250 CBCT scans of a mandibular first molar of an Egyptian population were collected from preexisting CBCT database of a private dentomaxilloficial radiology center, 90 scans were excluded as shown in (table no. 14).

Table (14): A table showing Different causes and the numbers of excluded teeth within the in-vivo study

The age out of range	**25**
Previously endodontic treated teeth	**19**
Teeth showing internal or external root resorption	**15**
Teeth showing pulp calcification and/or stones	**22**
Teeth have immature apecies.	**9**
Teeth have any type of root fracture or cracks.	**0**
Total	**90**

Regarding the middle mesial canal (MMC), the results of this in-vivo study revealed that the prevalence of MMC is 21.98 % (233 of 1060 total sample) in the tested CBCT scans of the individuals (Voxel size: 200-75 micron). this result was in conflict with that in-vivo result of Wang et al.[119] on a Western

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Chinese population that identified MMC in 2.2 % (12 of 410 total sample) using CBCT exploration method (voxel size: 125 micron) and also in conflict with that in-vivo result of Kim et al [120] on Korea population that identified MMC in 0.26 % (5 of 1952 total sample) using CBCT exploration method (voxel size: 167 micron). This discrepancy in the prevalence of the MMC when explored with CBCT scans may be attributed to the race differences in addition to the difference in methodology including the voxel size of each CBCT machine used.

Regarding the middle distal canal (MDC), the results of this in-vivo study revealed that the prevalence of MDC is 6.32 % (67 of 1060 total sample) in the tested CBCT scans of the individuals (Voxel size: 200-75 micron). Although upon searching, there was no available research found on the prevalence of MDC using CBCT exploration method

Regarding the correlative with age, the results revealed that there is no correlation between the prevalence of middle mesial and middle distal canal from one side and the age of the tested individuals.

Furthermore, there is no correlation between the prevalence of middle mesial and middle distal canal with the sex of individuals.

In conclusion, the null hypothesis of this study was accepted, as there is no difference among the tested exploration methods used in the study.

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Summary

The complexity of root canal anatomy and its variations such as the presence of extra root canals has a great significance on the endodontic outcome [4]. Therefore, the clinician should be aware of them to avoid a failure of endodontic treatment [1,2] Among the permanent dentition, the mandibular first molar is the first tooth that erupts, caries, and most of them need endodontic treatment [7].

Many techniques were used to examine the root canal anatomy. The old techniques have a lot of shortages like distortion and damage of samples, no more details gaining from it or superimposing of images and so on. On the other hand, the new techniques used to explore the root canal anatomy such as magnification “DOM”, three-dimensional image “CBCT and micro-CT”. These give good vision and details that make the clinician encounter the complex morphology [8-12].

In this study, the In-vitro study on mandibular first molars to explore the prevalence of middle mesial canal and middle distal canal in an Egyptian population using different methods “CBCT, DOM, clearing/staining exploration method” and In-vivo study using CBCT exploration method.

In an in-vitro study, found the prevalence of MMC in mandibular first molar using three methods; CBCT, DOM and clearing/staining methods as follows: 21.88%, 28.75%and 33.54%. Also, the prevalence of MDC in mandibular first molar using three methods; CBCT, DOM, and clearing/staining methods as follow: 6.88%, 8.125%and 7.45%. In MMC, there was no statistically significant difference between different in vitro methods CBCT, DOM, and clearing/staining. In MDC, there was no statistically significant difference between different in vitro methods CBCT, DOM and clearing/staining.

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From the above, we conclude that when using many techniques to explore extra canals, we get a better result.

In an in-vivo section, found the prevalence of MMC in mandibular first molar using CBCT scans was 21.98%. Also, the prevalence of MDC in mandibular first molar using CBCT was 6.32%. Moreover, there is no correlation between the occurrence of MM canal and age or between the occurrence of MD canal and age. Also, no statistically significant difference correlates with sex.

In conclusion, when using many techniques to explore extra canals, getting a better result and high success rate of endodontic treatment.

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Conclusion

- Both of cone beam computed tomography and dental operating microscope are effective methods for detecting middle mesial and middle distal canal
- The clearing/staining method remains the gold standerdd in comparison with both of the cone beam computed tomography and dental operating microscope with regarding to detection of middle mesial canal and middle distal canal.
- The perfect merge between cone beam computed tomography and dental operating microscope can improve the accuracy of detecting middle mesial and middle distal canal.
- Even magnification and CBCT are good methods to detect MMC & MDC in the mandibular first molar, none of them reached to the accuracy of the percentage clearing/staining method of the tested canals.

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Recommendation

- Further research should be regard the anatomical complexity in the mandibular first molar.

- More research should be done using the new techniques of exploration especially on mandibular molars.

- More effort should be done on dealing with accessory canals.

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