https://doi.org/10.35845/kmuj.2021.21653

ORIGINAL ARTICLE

USE OF TOOTH CLEARING TECHNIQUE TO DETERMINE ROOT AND CANAL MORPHOLOGY OF PERMANENT MANDIBULAR THIRD MOLARS IN POPULATION OF PESHAWAR: AN IN VITRO CROSS-SECTIONAL STUDY

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ABSTRACT

OBJECTIVE: To find out number of roots, root-canals and canal configuration in permanent mandibular third molars through tooth clearing technique.

METHODS: In this cross-sectional study, 193 extracted human mandibular permanent third molars with completely formed apical foramen and intact roots were collected from both genders treated at dental hospitals in Peshawar, Pakistan from 1st July to 31st December 2019. After collection teeth were visually inspected to count number of roots, followed by access cavity preparation, pulp extirpation and canal staining with black Indian ink. Decalcification was done by placing teeth in nitric acid for 5 days followed by dehydration in ascending concentrations of alcohol. Complete transparency was achieved by immersing teeth in methyl-salicylate for 72 hours. Transparent teeth were inspected again for number of roots and root-canals.

RESULTS: Among 193 extracted mandibular third molars, (n=161; 83.4%) had two-roots and (n=24; 12.4%) were single-rooted. Two-canals were present in vast majority (n=142; 73.6%) whereas three and one-canal were seen in (n=37; 19.2%) and (n=13; 6.7%) teeth respectively. Most common type of root canal pattern was Vertucci's Type-I in mesial-roots (n=79; 63.7%) and distal-roots (n=120; 96.8%). Vertucci's Type-II and Type-IV were (n=15; 12.1%) and (n=12; 9.7%) in the mesial-roots respectively. Mandibular third molars didn't present with any configurations that didn't fullfill Vertucci's criteria. Correlation between number of roots and root-canals of mandibular third molars was non-significant.

CONCLUSION: Two-roots and two-canals were common patterns for mandibular third molars. Mesial and distal roots were predominant in Type-I followed by Type-II and Type-IV Vertucci's classification.

KEY WORDS: Canal configuration (Non-MeSH); Clearing technique (Non-MeSH); Molar, Third MeSH); Vertucci's classification (Non-MeSH); Dental Pulp Cavity (MeSH); Tooth Root (MeSH).

THIS ARTICLE MAY BE CITED AS: Khattak I, Khattak MA, Khattak YJ, Arbab S, Rashid M, Shah SA. Use of tooth clearing technique to determine root and canal morphology of permanent mandibular third molars in population of Peshawar: an in vitro cross-sectional study. Khyber Med Univ J 2021;13(4):216-21. https://doi.org/10.35845/kmuj.2021.21653.

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Date Submitted: June 1, 2021

Date Revised: September 24, 2021

Date Accepted: September 28, 2021

INTRODUCTION

Permanent mandibular third molars erupt at the age of seventeen to twenty-one years.[1] Its eruption age varies in different races,[2] and its function complements the other molars in grinding.[1]

Third molars are routinely extracted due to caries, pericoronitis, gingival and periodontal diseases.[3] In modern dentistry they have been used in auto transplantation as a replacement for hopeless first and second molar teeth.[4,5] Prior knowledge regarding shape, number and positions of roots may be advantageous for easy and less traumatising extractions following successful root canal therapy in autotransplantation.[6] Hence, a detailed knowledge regarding the complex root structure of third molars is very important for a successful endodontic therapy[7] because the complication of the root canal apparatus is the utmost task for any dental practitioner planning root canal procedure, therefore insufficient understanding about root canal system may result in treatment failure , even though the basic principles of endodontics are followed.[8]

The structure of mandibular third molars has been reported as uncertain,[9] since root morphology may vary among different population groups.[10] This structural disparity may be an outcome of ethnicity, chronological age, and sex.[11] It has been found that hereditary elements influence the root structure and a diversity of root canal arrangements in distinct individuals, hence it is mandatory to evaluate the root form in separate ethnic groups.[12]

The common mandibular third molar may have 1-4 roots, with numbers of encased root canals varying from 1-6, still the number varies according to different studies.[13,14] Kuzekanani M, et al.[15] reported from Iran that the bulk of sample (73%) had two roots, (21%) were single rooted and 5.5% of the sample had three roots. In 2016 Ahmad I, et al.[16] carried a study using the clearing technique in a Jordanian population using 159 extracted third molars. He reported that 74.3% of lower third molars presented with 2

TABLE I: DISTRIBUTION OF THE MANDIBULAR THIRD MOLAR TEETH ON THE BASIS OF THE NUMBER OF ROOTS AND ROOT CANALS

Number of roots & root canals		Number of Teeth (n=193)	Percentage (%)
Number of roots	1	24	12.4
	2	161	83.4
	3	7	3.6
	4	1	0.5
Number of root canals	1	13	6.7
	2	142	73.6
	3	37	19.2
	4	1	0.5

roots and 2 (38.6%) and 3 (45.7%) canals. Razumova S, et al.[9] also reported that mandibular third molar is found frequently with 2 roots, a mesial and a distal root with 2 or 3 canals. Most of the previous authors not only worked on the external form of mandibular third molar but also explained the internal form applying mostly the Vertucci's classification (1984). It was reported from a Jordanian population that the mandibular third molars showed Type- I configuration in distal roots (94.3%) and a maximum percentage of mesial roots (56%) had 2 canals out of which Type- IV and Type- II were the most dominant configurations (26.5% and 20.6%, respectively).[16] And lastly in 2019, few authors using CBCT from Russia reported that out of a sample of 210 mandibular third molars, majority were Type- I for the mesial and distal roots of two and single rooted specimens.[9]

On extensive literature review, no local research has been done on the figure of roots, canals, and canal morphologies in permanent mandibular third molar teeth. This investigation will evaluate the number of roots, canals, and canal shape in lower third molars in patients reporting at the three dental teaching hospitals in Peshawar.

The results obtained from this study will be helpful in establishing a baseline data about the root canal system of mandibular third molars. Also, the clinicians will have a very good knowledge about the anatomy of third molars while performing endodontic procedures. This will aid in saving third molars from being extracted in cases where it can help in conservative and less costly fixed prosthesis in comparison to the costly implants in future.

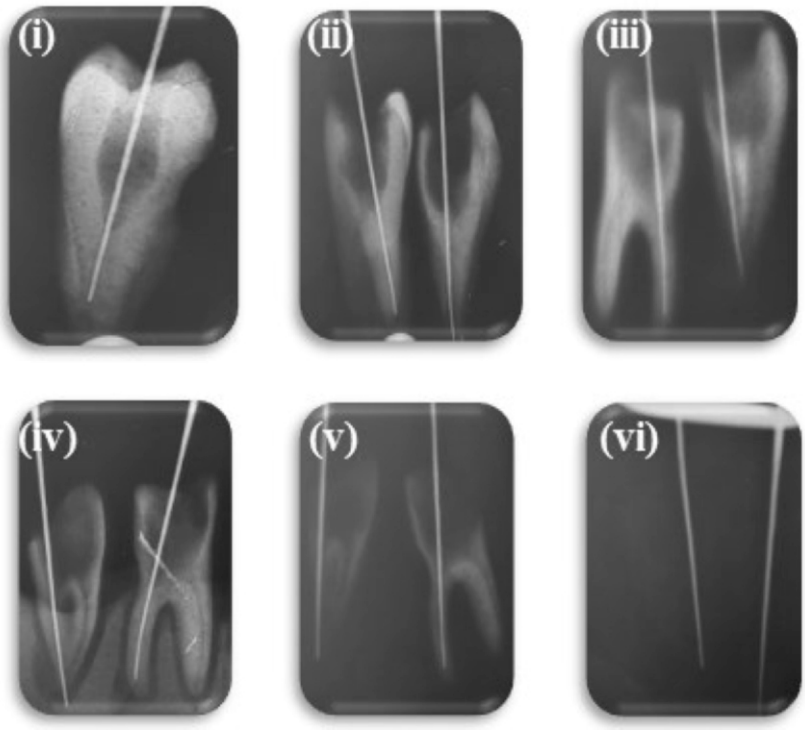

Figure 1: Radiographs taken to assess the process of demineralization post nitric acid (HNO3) decalcification after 24 hrs each. i) Before Nitric Acid treatment at (0 hours) ii) At 24 hours iii) At 48 hours iv) At 72 hours v) At 96 hours vi) At 120 hours

METHODS

In this in-vitro cross sectional study, a total sample of 193 extracted permanent mandibular third molar teeth were collected from the Department of Oral and Maxillofacial Surgery of Peshawar Dental College, Khyber College of Dentistry and Sardar Begum Dental College and Hospital - Peshawar - Pakistan via non-probability convenience type sampling from patients treated at the hospital after taking prior permission from 1st July 2019 to 31st December 2019. Patient's details and informed consent were taken on pre-structured data sheets before collecting the teeth for research purpose.

The teeth were washed gently with tap water to remove any blood or food debris. If any dental calculus or soft tissue remnants being adhered to any tooth, were removed using an ultrasonic scaler. The samples were stored in 5.25% sodium hypochlorite (NaOCL) for half an hour for the dissolution of organic matter after extraction. Following wash in running tap water, each tooth was then placed in a small, labelled plastic bottle that contained 10% formaldehyde and were stored until a total sample of 193 mandibular third molars were collected.

After completion, all teeth were visually examined to count the number of roots. The sample of the extracted third molars were processed with the application of tooth clearing technique to know the number of canals and configurations according to Vertucci's criteria. Before use each tooth was cleaned in normal tap water, disinfected, and dried at room temperature. Cavities were drilled and the pulp substance was removed. The individual teeth were again dipped in 2.5% NaOCL solution overnight to liquefy any pulpal residues. The samples were again cleaned in tap water for 2 hours, dried at room temperature and black Indian ink was administered in the root canals of each tooth via 27-gauge needle

TABLE II: DISTRIBUTION OF THE MANDIBULAR THIRD MOLAR TEETH ON THE BASIS OF THE ROOT CANAL CONFIGURATION (VERTUCCI'S CLASSIFICATION)

No. of teeth (n)	Root morphology		Vertucci's Canal Configuration							
			(1-1) % (n)	(2-1) % (n)	(1-2-1) % (n)	(2-2) % (n)	(1-2) % (n)	(2-1-2) % (n)	(1-2-1-2) % (n)	(3-3) % (n)
24	Single root		45.8 (11)	29.2 (7)	8.3 (2)	12.5 (3)	-	4.2 (1)	-	-
124	Two separate roots	MR	63.7 (79)	12.1 (15)	4.8 (6)	9.7 (12)	8.9 (11)	-	0.8 (1)	-
		DR	96.8 (120)	-	1.6 (2)	-	1.6 (2)	-	-	-
37	Two fused roots		-	13.5 (5)	-	75.7 (28)	-	-	-	10.8 (4)
5	Three separate roots	MBR	100 (5)	-	-	-	-	-	-	-
		MLR	100 (5)	-	-	-	-	-	-	-
		DR	100 (5)	-	-	-	-	-	-	-
2	Three fused roots		-	-	-	-	-	-	-	100 (2)
1	Four separate roots	MBR	100 (1)	-	--	-	-	-	-	-
		MLR	100 (1)	-	-	-	-	-	-	-
		DBR	100 (1)	-	-	-	-	-	-	-
		DLR	100 (1)	-	-	-	-	-	-	-

MR: Mesial root, DR: Distal root, MBR: Mesiobuccal root, MLR: Mesiolingual root, DBR: Distobuccal root, DLR: Distolingual root.

and syringe. Each tooth was kept vertically in packing material for 24 hours allowing the ink to leak from the apical foramen and dry properly staining the canals outline. After the ink had dried, each tooth was stored individually in a labelled container for demineralization for 5 days in 5% nitric acid (HNO_3) solution. The HNO_3 solution was replaced with fresh solution every 24 hours and the process of decalcification and demineralization was monitored radiographically by the placement of a file in the crown and root canals (Figure 1). Following demineralization, each tooth was washed under tap water for continuous 4 hours and then placed in ethyl alcohol preparations of 70%, 80% and 95% for dehydration for 24 hours each. At the completion of this duration, there was complete loss of opacity. Finally, by immersing the teeth in methyl salicylate, complete transparency was achieved at the end of 72 hours and clearing procedure was rendered complete.

Number of canals and root canal morphology of the cleared teeth were visualised with the naked eye using good lighting and a magnifying glass of (3X) magnification.[16] Data was recorded in the pre-structured data collection sheets.

Root canal morphology was documented and categorized using Vertucci's system for classification of root canal morphology.

The statistical analyses were performed using SPSS software (version 25). A relationship between the number of roots and root canals was demonstrated using the Pearson Chi-Square test. Variations, if any, from the mean values were considered statistically significant when the p-value was < 0.05.

RESULTS

A total of 193 extracted human mandibular third molar teeth were assessed for the presence of the number of roots, root canals and canal configurations. They were collected from both male (48.7%) and female (51.29%) patients.

One hundred and sixty-one mandibular third molars (83.4%) had two roots compared to 24 (12.4%) having a single root (Table I). Similarly two canals were observed in 142 (73.6%) mandibular third molars, followed by three canals found in 37 (19.2%) teeth (Table I).

The two rooted specimens were the most prevalent (n=161; 83.4%) out of which two separate and two fused were in the order of 77% and 22.9% respectively. Type-I (1-1) was in majority, mostly in mesial roots (n=79; 63.7%) and distal roots (n=120; 96.8%) of two separate root third molars and in a single rooted (n=11; 45.8%) mandibular third molars. Followed by Type-II (2-1) and Type-IV (2-2) canal configurations in the order of (n=15; 12.1%) and (n=12; 9.7%) for the mesial roots of two separate and (n=5; 13.5%) and (n=28; 75.7%) for the two fused rooted mandibular third molars respectively.

Single rooted specimens showed a range of configurations with Type I (1-1) 45.8% being the predominant type followed by Type-II (2-1) 29.2% and Type-IV (2-2) 12.5%. Few molars (n=4; 10.8%) in the two fused forms and all three fused rooted (n=2; 100%) had Type-VIII (3-3) canal configuration as shown in Table II.

Samples of root canal configurations observed are given in figure 2.

Comparison of number of roots and root canals of mandibular third molars showed no statistical significance (Table III).

DISCUSSION

Precise knowledge of the exterior and interior structure and form of teeth along its anatomical variations is necessary for a successful dental treatment.[9,14] With correct diagnosis and planning, endodontically involved third molars may

TABLE III: RELATION BETWEEN THE NUMBER OF ROOTS AND ROOT CANALS OF MANDIBULAR THIRD MOLAR TEETH

Number of roots	Number of teeth "n" (%)				Total (n=193)	df	*P-Value
	1RC	2RC	3RC	4RC			
1	2 (8.3%)	19 (79.2%)	3 (12.5%)	0	24 (12.43%)	9	0.538
2	11 (6.83%)	119 (73.9%)	30 (18.6%)	1 (0.62 %)	161 (83.4%)		
3	0	4 (59.1%)	3 (42.9%)	0	7 (3.6%)		
4	0	0	1 (100 %)	0	1 (0.51%)		
Total	13 (6.7%)	142 (73.6%)	37 (19.2%)	1 (0.5%)	193 (100%)		

*chi square applied, RC: Root canal. "P" value= 0.538, df= 9 as calculated by Pearson Chi Square Test.

effectively be restored and preserved as an effective and useful element in the mandibular arch. [17]

In the present study clearing technique has been used for studying the internal anatomy of roots as it was less costly, did not require any modern, complex, and expensive armamentarium. Following rendering the teeth transparent, the actual path of the root canal system was easily studied all the way beginning at the canal opening to the apical foramen to yield accurate canal configuration. Same methodology has been adopted by several researchers for the evaluation of root canal morphology and patterns for the third molars of both the arches. [13-16, 18-20]

In the present study 83.4% mandibular third molars demonstrated two roots which was certainly lower than the findings (100%, 86.7%) of Burmese and Thai population respectively. [18,21] Both the studies were done using the clearing technique. The two rooted mandibular third molars were higher than those seen in Jordanian population (74.3%) [16] and in Iranians (72.6%) and Turkish population (69.5%) respectively. [15,22] The incidence of 83.4% two rooted mandibular third molars hold the results of Razumova S, et al. (2018) who showed that the Russian natives had a prevalence of 80% for two rooted mandibular third molars and 77% in a group of Americans. [23]

The prevalence of single root mandibular wisdom molars in the present study was 12.4% which was in accordance with (11.6%) in Thai population [21] but was lower than the findings of (56%, 47.7%, 24.9%, 21.4% respectively). [15,17,22,24] The dissimilarity in findings may be due to different methods used, sample size and race variations amongst different populations. Similarly, three and four rooted variants were the least in the order of (3.6%) and (0.5%) sequentially in the present study.

The current work reported two canals to be present in 73.6% mandibular third molars. This incidence was considerably higher than the studies reported in a group of U.S inhabitants (16.7%), Croatians (6%), Jordanians (38.6%) and Russians (40.9%). [9,16,23,24] The Incidence of two canals (64.1%) showed similarity to the work of Gulabivala K, et al. [21] Furthermore, three canals were found in 19.2% teeth. This occurrence was much lower than those of earlier studies [9,24] but in accordance with the findings (17.3%) of Sert S, et al. [22]

Single canal ranged from 0.5% in one study [9] to 10.8% in another study. [22] In the current work, 6.7% of lower third molars had single canals which was in consistence to the work done by Gulabivala K, et al. (6.4%). [21]

In the current study, Vertucci's classification [20] was made use of in its original form with no modifications because it is the only criteria that considered both the canal orifice and apical foramina simultaneously. Also, most of the researchers who practised the in vitro clearing technique used it for determining canal configurations. [15,16,21-23]

In the present study, the findings were interestingly similar with the aforementioned studies. Vertucci Type -I was the predominant form as previously reported. [16,23,25]

The single rooted variant showed a range of canal configurations with Type-I being the most common form (45.8%) in the current study, which was in accordance (65.6%, 31.2%, 55.6%, 42.7%) to the previous studies [15-17,22] that reported Type-I being the most prevalent canal configuration in single rooted mandibular third molars. The incidence of Type-II (29.2%) and Type-IV (12.5%) in single rooted teeth were in accordance with the previous studies mentioned.

The mesial root of two separate rooted lower third molars were predominant in the present study with Type-I (63.7%) canal configuration followed by Type-II (12.1%) and Type-IV (9.7%) which was in accordance with the work published by Sert S, et al. 2011(59%), Kuzekanani M, et al. 2012 (54.1%) and Zhang W, et al.2018 (68.3%) for the Type-I canal configuration. Similarly, for the distal root of two separate rooted

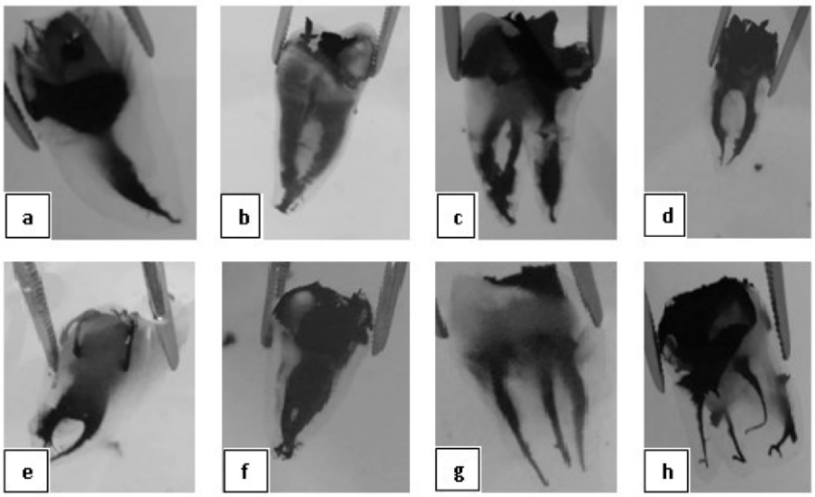

Figure 2: Sample of Root Canal Configurations observed in this study. (a) Type I (1-1), (b) Type II (2-1), (c) Type III (1-2-1), (d) Type IV (2-2), (e) Type V (1-2), (f) Type VI (2-1-2), (g) Type VIII (3-3), (h) Type 4-4.

specimens were likewise prevalent in Type-I canal shapes (96.8%) in the current study which was in accordance with (99.2%, 92.7%, 93.8%, 100% respectively) as reported previously. [15-17,22] The two fused rooted teeth were predominant in Type-IV (75.7%) which agreed with the results of Ahmad et al. 2016 (45%). [16]

In our study, there was a high statistically non- significant difference ($p>0.05$) when the number of roots of mandibular third molar teeth were compared with their root canals. It was noted in this study that the mesial root of mandibular third molar teeth displayed greater variation in the number of root canals among the two and three rooted groups. This finding was supported by the result of one of earlier study published in the literature.[9]

The current study has its own advantages i.e., it is economical and convenient not requiring expensive and advanced technical facility and tools and provides a baseline for advanced studies. It however has certain limitations i.e., it is an in-vitro study, so it cannot help the clinicians in detecting the atypical morphology of the intact mandibular permanent third molars inside the oral cavity. The use of modern dental tools was lacking i.e., the dental operating microscope.

CONCLUSION

Mandibular third molars of the investigated population of Peshawar revealed a variety of root and canal patterns. Mandibular third molars predominantly had two roots including two separate and two fused roots forms. The two rooted variant with two root canals i.e mesial and distal canal was the common and predominant form occurring followed by three root canals to a limited extent. Vertucci's Type- I (1-1) was the most typical and usual root configuration in both the roots of mandibular third molars followed by Type- II (2-1) and Type- IV (2-2) in the mesial roots.

ACKNOWLEDGMENT

The authors would like to thank Dr. Sajid Hussain, Assistant Professor, Department of Dental Materials, Frontier Dental College (Abbottabad, Pakistan) for his volunteer assistance in statistical analysis of this research.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

IK: Conception and study design, analysis, and interpretation of data, drafting the manuscript, critical review, approval of the final version to be published

MAK: Conception and study design, critical review, approval of the final version to be published.

YJ: Acquisition of data, drafting the manuscript, approval of the final version to be published.

SA: Analysis and interpretation of data, critical review, approval of the final version to be published

MR: Acquisition, analysis, and interpretation of data, drafting the manuscript, approval of the final version to be published.

SAS: Study design, drafting the manuscript, critical review, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE

Authors have declared no specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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