Establishing legal threshold of 18-years based on the assessment of mandibular molars using three different methods – An observational study

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ABSTRACT

Background: The study evaluates the feasibility of employing the radiographic visibility of the root pulp and periodontal ligament in mandibular molars for age estimation, particularly focusing on the 18 years of age threshold. This study additionally investigates the potential of root canal width reduction in mandibular molars, as a reliable method for forensic age estimation in living individuals.

Materials and methods: A cross-sectional study was conducted to assess the radiographic visibility of the root pulp (RPV) and the root canal width (RCW) of mandibular first, second, and third molars along with the radiographic visibility of the periodontal ligament (PLV) of mandibular third molars, in a sample of 403 individuals aged 16-25 years (220 males and 183 females). Data regarding age for different stages of RPV and PLV and various types of RCW were recorded and observed for sex-based differences. Results obtained were tabulated and descriptive statistics were applied to summarise the findings.

Results: Individuals over 18 years old were classified with higher accuracy using stage 3 of the RPV scoring system in all mandibular molars (first, second, and third) compared to stage 2, which was also effective for the second and third molars. This result held regardless of sex and side examined. Additionally, root canal width (RCW) assessment demonstrated that individuals with RCW types A, B, and C were more likely to be under 18 years old in both sexes. Conversely, individuals with RCW type U on the right side for males and the left side for females exhibited a higher likelihood of being above 18 years old.

Conclusion: The study suggests that the assessment of mandibular molars could potentially serve as an auxiliary tool in age estimation methods, particularly for approximating individuals around the 18 years of age threshold. Further investigation is warranted to explore the potential application of root canal width measurements in forensic age estimation.

INTRODUCTION

Estimating the age of individuals, whether alive or dead, is becoming increasingly significant for forensic purposes because it narrows down the search for the missing person.[1] Age estimation has a wide range of interests and objectives because it necessitates a multi-disciplinary effort to assist in forensic and medico-legal issues. Forensic age estimation is becoming

increasingly important in criminal and civil processes, individual identification, immigration, and competitive sports. [2, 3]

Dental age (DA) correlates with chronological age (CA) more closely than any other human biological growth marker (HBGM). [4] Dental maturation refers to the complicated sequences of events that occur during the formation of a crown, root development, eruption of the tooth into the oral cavity, and root apex maturation. Tooth development is a more accurate indicator of dental maturity than tooth eruption. [5] Therefore, examining such occurrences clinically or radiographically can aid in determining CA, which is useful in a variety of fields, including paediatric dentistry, orthodontics, forensic odontology, and human anthropology. [6] Because it is straightforward, non-invasive, and repeatable, radiographic examination is commonly used to determine dental maturity and age (DA). [7, 8]

According to ABFO, orthopantomograms (OPGs) are a component of dental examination and are thus frequently used in the forensic field. OPGs and periapical radiographs show the morphology of posterior tooth roots, the shape and volume of the pulp chamber in teeth, and the relationship between third molars and the surrounding mineralised structures. Probable identification and age determination of a person, ante-mortem, and post-mortem radiographs of teeth can be used to detect caries, bone loss, endodontic treatment, crown and bridge restorations, etc. [9, 10]

Despite their developmental variability, third molars are the most accurate biological indicator of age in adolescents. [11] The agenesis of the third molar is the most common issue when using this tooth to estimate age. [12] When the third molar is congenitally absent or extracted, another approach using teeth other than the third molar [13, 14] would be adequate for discriminating whether a person has reached maturity or not.

Dental age estimation in individuals above 15 years of age and suspected to be older than 18 years is a growing area of concern in forensic science, and age estimation using teeth is accurate up to approximately 15 years of age. [15] In the absence of third molars and other reliable age indicators, it has been proven that mandibular second molars can be used to determine whether the age of an individual is above 18 years or not. [13]

Concerning the legal age of an individual, 18 years is significant because anyone under that age is considered a minor. Since identifying the 18 years of age threshold to distinguish between the status of a minor and an adult is a considerable risk, the proof of achievement of 18 years is of the highest importance in the age estimation of live persons. [16, 17] Two maturity markers, root pulp visibility [18] and periodontal ligament visibility, [18, 19] were commonly used to identify whether an individual had attained 18 years of age or not.

Obliteration or interruption of the root pulp and periodontal ligament develops as age increases because these growth indicators appear to be dependent on mandibular bone development. This led to the discovery of another HBGM in which the relative widths of the distal root canals (RCW) of the lower permanent molars (LL6, LL7, and LL8) were assessed. [20] Based on their evaluation in an orthopantomogram, Roberts et al. [20] divided the relative widths into three stages, namely: RCW-A, RCW-B, and RCW-C.

Owing to the scarcity in the literature, the present study assesses the mandibular molars for age estimation using three methods. It aims to determine whether a threshold of 18 years of age can be obtained based on the radiographic assessment of the visibility of mandibular molars.

MATERIALS AND METHODS

A retrospective analysis was conducted using digital orthopantomograms (OPGs) obtained from the archives of the Department of Oral Medicine and Radiology at a private institution. The OPGs were originally collected for treatment planning and other clinical purposes from 403 individuals aged 16-25 years. Informed consent was obtained at the time of taking the radiograph indicating that it will be used for research purposes while maintaining its confidentiality. This retrospective observational study spanned a period of 12 months from June 2021 to June 2022. It was carried out after obtaining ethical clearance from the Institutional Ethical Committee following the ethical standards set by the Declaration of Helsinki (Finland). [21]

OPGs with the presence of lower right and left mandibular molars (first, second, and third molars) that had root apices closed (Demirjian developmental stage H) and radiographs with no evidence of gross pathology affecting the mandible were included. OPGs that do not depict the area of interest with missing molars on either side due to extraction or congenital

absence and those with any systemic disease or any disorder that affects tooth development are excluded from the study. Also, mandibular molars with existing root canal restorations, single roots, or any evidence of infection e.g. pulpitis, periodontitis, etc. are excluded from the study.
OPGs that met inclusion criteria were exported after masking the demographic details of name, age, and gender in a Tagged Image File Format (tiff) and assigned a random number to ensure blinding for the observers. Two observers (MS and JP) were calibrated for Olze et al., [18, 22] and Roberts et al., [20] methods by staging a sample of 10 OPGs that were not part of the final analysis, and assessment of the sample was provided only after obtaining standardisation between them. Demographic details of the included samples are described in Table 1.

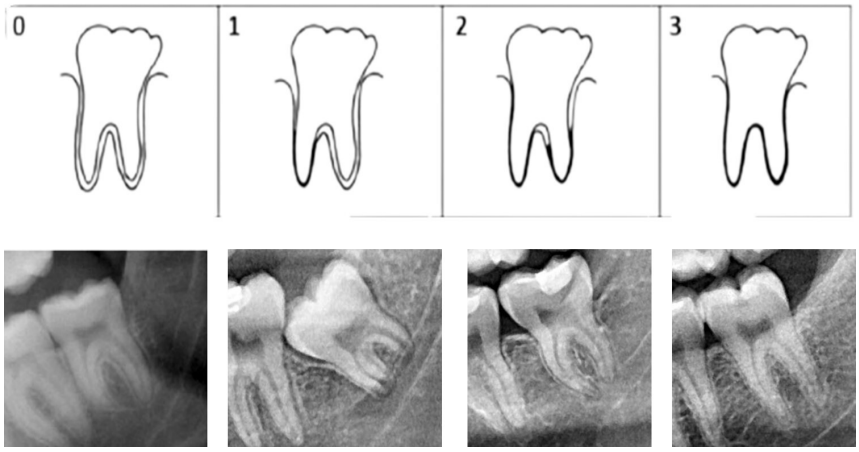
Table 1. Demographic details of the participants described in terms of number and percentage

Category			n	%
Gender		**Male**	183	45.4
		Female	220	54.6
Age Group (Years)	16-16.9	**Male**	7	3.83
		Female	14	6.36
	17-17.9	**Male**	9	4.92
		Female	15	6.82
	18-18.9	**Male**	24	13.11
		Female	19	8.64
	19-19.9	**Male**	19	10.38
		Female	30	13.64
	20-20.9	**Male**	19	10.38
		Female	34	15.45
	21-21.9	**Male**	25	13.66
		Female	29	13.18
	22-22.9	**Male**	28	15.30
		Female	27	12.27
	23-23.9	**Male**	12	6.56
		Female	19	8.64
	24-24.9	**Male**	40	21.86
		Female	33	15.00
Gender			**Mean**	**SD**
		Male	21.54	2.72
		Female	21.20	2.71
		Total	21.35	2.72

Olze Method of radiographic visibility of root pulp and periodontal ligament
Olze et al., [18] were first to devise a classification system based on radiographic visibility of root pulp in mandibular third molars. He devised a four-stage system based on the visibility of the lumen in the root canals on an orthopantomogram as depicted in Figure 1.

1. Stage 0: Visibility of the lumen is noted to the apex in all the canals.
2. Stage 1: Visibility of the lumen is not till the apex in one of the canals.
3. Stage 2: Visibility of the lumen is not till the apex in both the canals or one of the canals is virtually invisible at full length.
4. Stage 3: Visibility of the lumen is completely absent in both canals.

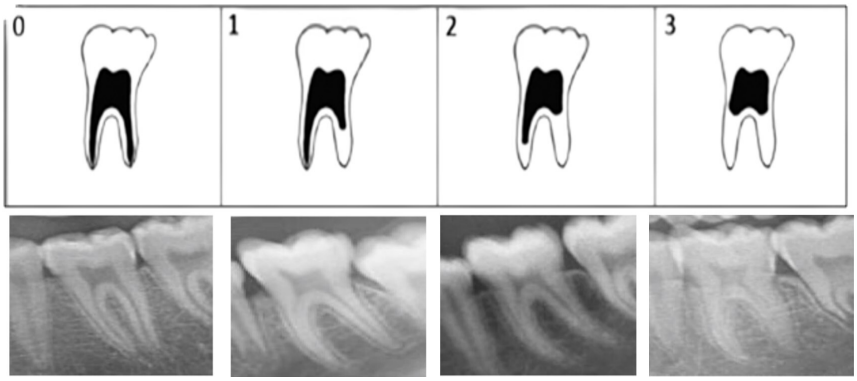
Figure 1. Radiographic visibility of the root pulp according to stages devised by Olze et al., [18] depicted in the mandibular left first molar.



Later, Olze et al., [22] classified the radiographic visibility of periodontal ligament in completely mineralised mandibular third molars. A four-stage system similar to the radiographic visibility of root pulp was utilised as depicted in Figure 2.

1. Stage 0: Visibility of the periodontal ligament is noted along the complete length of the root.
2. Stage 1: Visibility of the periodontal ligament is partially obliterated in one root and from apex till more than half of the other root.
3. Stage 2: Visibility of the periodontal ligament is obliterated along the entire length of one root or the part of the root in both roots.
4. Stage 3: Visibility of the periodontal ligament is completely obliterated in both canals.

Figure 2. Radiographic visibility of the periodontal ligament according to stages devised by Olze et al., [22] depicted in the mandibular left third molar



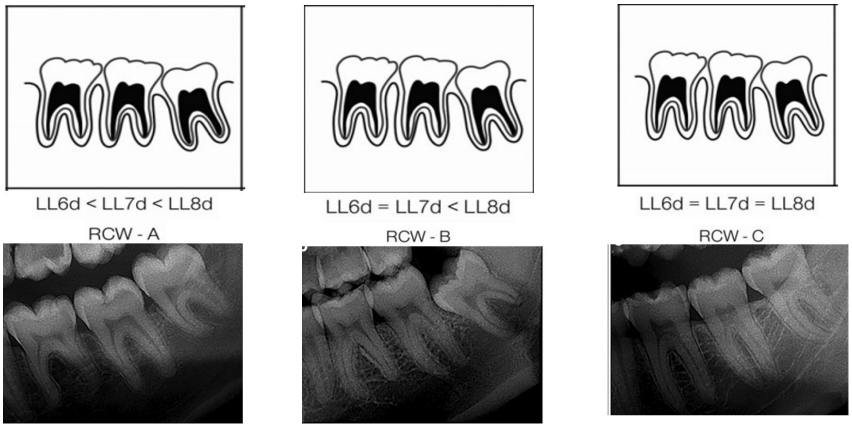
Roberts method:

Roberts et al., [20] proposed an identification marker based on the mesio-distal widths (m-d) of the root canals of left mandibular molars. The distal root canals of the mandibular first, second, and third molars were considered a pattern in predetermined 3 stages as depicted in Figure 3.

1. RCW-A (Root canal width - A): The m-d width of the mandibular first molar (LL6) is narrower than the m-d width of the mandibular second molar (LL7) which in turn is narrower than the m-d width of mandibular third molar (LL8), read as LL6 < LL7 < LL8.
2. RCW-B (Root canal width - B): The m-d width of the mandibular first molar (LL6) is equal to the m-d width of the mandibular second molar (LL7) which in turn is narrower than the m-d width of the mandibular third molar (LL8), read as LL6 = LL7 < LL8.
3. RCW-C (Root canal width - C): The m-d width of the mandibular first molar (LL6) is equal to m-d with of mandibular second molar (LL7) which in turn is equal to the m-d width of the mandibular third molar (LL8), read as LL6 = LL7 = LL8.

4. RCW-U (Root canal width – U): Root canal patterns that met the inclusion criteria and did not fall under any of the stages mentioned above are classified as unknown.

Figure 3. Radiographic and schematic representation of the three categories described by Roberts et al., [20] Abbreviations RCW Root canal width LL6 Mandibular left first molar LL7 Mandibular left second molar LL8 Mandibular left third molar RL6 Mandibular right first molar RL7 Mandibular right second molar RL8 Mandibular right third molar



To assess repeatability and inter-observer agreement, two observers (MS and JP) evaluated all the OPGs. To assess intra-observer agreement, one observer (MS) observed 100 OPGs in a random sample twice after 6 months. The three methods mentioned above were applied to obtain dental ages, which were then statistically assessed after being imported into Microsoft Office Excel 2016.

Statistical analysis:

The Statistical Package for the Social Sciences (SPSS) 21.0 for Windows was used for statistical analysis (SPSS Inc., Chicago, IL, USA). The significance level for analysis was set at 5% ($p < 0.05$). Individual age for each sample was calculated before anonymisation by an observer (LK) who was not part of the assessment. The date of exposure was subtracted from the date of birth and it was recorded as chronological age. Mean, standard deviation, minimum, median, maximum, lower quartile, and upper quartile were determined for root canal visibility, periodontal ligament visibility, and root canal width wherever applicable. Also, the applicability of these methods in classifying the individuals who have attained major status (≥ 18 years) was determined. To evaluate intra-observer and inter-observer reliability, Cohen's kappa statistics were applied.

RESULTS

Age-related trend changes are noted similarly in the mandibular first, second, and third molars on the left side of males and females. Inter-examiner reliability for radiographic visibility of the root canal of the first, second, and third mandibular molars on both sides was 0.84, 0.86, and 0.82, respectively. Intra-examiner reliability revealed that radiographic visibility of the root canal was 0.82, 0.84, and 0.83, respectively for mandibular first, second, and third molars. Cohen's Kappa values for radiographic visibility of the periodontal ligament of third molars were 0.84, and 0.82 for inter- and intra-observer reliability, respectively. Based on the results obtained, substantial agreement was noted for assessed parameters, thereby confirming its reliability.

The study found a complete absence of Stage 0 (no radiographic visibility of root pulp) in both mandibular first molars and second molars of males, as well as mandibular first molars of females. Notably, the minimum age for mandibular first, second, and third molars in males did not exceed 16 years. However, intriguingly, stages 0 and 3 were not observed until after 19 years of age in mandibular second molars. Additionally, a consistent trend was observed across both sexes, with a gradual increase in participant age as the stage of root canal visibility progressed (higher stages indicating more closure). Detailed information on mean, minimum, and maximum values for root canal visibility stages in mandibular left first, second, and third molars for both males and females are presented in Table 2.

Table 2. Radiographic visibility of root canal in mandibular first, second, and third molars for both sexes on the left side Abbreviations: N – Number of participants SD – Standard Deviation

Sex	Tooth	Stage	N	Mean	SD	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Males	36	1	54	20.72	2.61	16.56	18.66	20.31	22.46	27.37
		2	87	21.56	2.63	16.11	19.44	21.14	23.07	27.31
		3	42	22.57	2.78	16.46	20.36	22.73	24.16	28.26
	37	1	122	21.16	2.70	16.11	18.76	21.23	22.71	27.37
		2	42	21.91	2.47	16.46	20.16	21.71	23.54	28.26
		3	19	23.22	2.82	16.61	21.80	24.07	25.43	26.48
	38	0	6	18.02	1.52	16.56	17.06	17.93	18.05	20.84
		1	10	17.83	1.19	16.11	16.77	18.09	18.45	19.58
		2	32	19.70	1.78	16.99	18.43	19.17	20.89	24.86
		3	135	22.41	2.46	16.88	20.66	22.47	24.43	28.26
Females	36	1	72	20.46	2.57	15.76	18.87	20.16	21.83	27.97
		2	105	21.28	2.81	15.82	20.06	20.91	22.59	28.75
		3	43	22.22	2.39	17.55	20.59	22.34	23.68	27.86
	37	0	3	20.40	1.32	19.27	19.68	20.08	20.97	21.85
		1	140	20.78	2.79	15.76	18.91	20.32	22.42	28.75
		2	58	21.66	2.53	16.59	19.56	21.87	23.22	27.86
		3	19	22.94	1.95	19.14	21.76	22.58	24.49	26.46
	38	0	26	18.46	1.46	15.76	17.47	18.52	19.07	21.11
		1	25	18.54	1.65	15.82	17.15	18.88	19.46	21.85
		2	50	21.05	1.72	17.04	20.18	21.19	22.05	24.21
		3	119	22.41	2.61	16.89	20.31	22.37	24.41	28.75

On the contra-lateral side, the study similarly documented an absence of stage 0 radiographic visibility of the root canal in both first molars, regardless of sex, and in male mandibular second molars. The minimum age for all evaluated male subjects was consistently below 18 years (<17 years) across all teeth and stages examined.

Conversely, female subjects exhibited a minimum age below 18 years for all stages in mandibular first and third molars, except stages 1 and 3 in mandibular second molars, where the minimum age exceeded 18 years. Mean values of root canal visibility along with minimum and maximum values for females are presented in Table 3.

Table 3. Radiographic visibility of root canal in mandibular first, second, and third molars for both sexes on the right side Abbreviations: N – Number of participants SD – Standard Deviation

Sex	Tooth	Stage	N	Mean	SD	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Males	36	1	56	20.61	2.57	16.56	18.45	20.15	22.41	26.10
		2	95	21.72	2.60	16.11	19.79	21.71	23.49	27.37
		43	32	22.65	2.91	16.61	20.25	22.77	25.22	28.26
	47	1	136	21.29	2.67	16.11	19.03	21.36	22.82	27.37
		2	36	21.86	2.67	16.61	19.83	21.94	23.54	28.26
		3	11	23.64	2.83	18.61	22.62	25.03	25.47	26.48
	48	0	8	17.74	1.45	16.11	16.74	17.78	18.02	20.84
		1	7	17.72	0.75	16.46	17.27	17.92	18.35	18.46
		2	38	20.08	2.10	16.61	18.65	19.68	21.31	25.61
		3	130	22.41	2.45	17.19	20.68	22.44	24.49	28.26
Females	46	1	63	20.41	2.69	15.76	18.58	20.16	21.84	26.78
		2	116	21.18	2.64	15.82	19.46	20.90	22.59	28.75
		3	41	22.44	2.57	17.55	20.33	22.60	24.04	28.08
	47	0	2	19.68	0.57	19.27	19.47	19.68	19.88	20.08
		1	144	20.66	2.71	15.76	18.83	20.32	22.34	28.75
		2	58	21.99	2.45	17.20	20.20	21.90	23.28	27.86
		3	16	23.31	2.18	19.14	22.19	23.52	25.47	26.46
	48	0	28	18.48	1.63	15.76	17.19	18.52	19.39	21.75
		1	23	18.57	1.60	16.02	17.39	18.34	19.46	21.85
		2	55	20.61	1.78	17.04	19.22	20.79	21.83	24.21
		3	114	22.67	2.46	16.89	20.80	22.47	24.61	28.75

In the current investigation, the radiographic visibility of the periodontal ligament in mandibular third molars was evaluated, as these teeth are the only ones still developing within the included age cohorts. In males, a progressive increase in minimum age was observed with advancing PDL visibility stages. Stage 1 visibility in the right mandibular third molar was associated with an age exceeding 18 years. Conversely, females exhibited stage 3 visibility in

the right mandibular third molar as the initial indicator of being above 18 years. A similar trend of increasing age with advancing PDL visibility stages (0-3) was observed in females. Detailed descriptive statistics (mean, standard deviation, lower and upper quartiles) are presented in Table 4.

Table 4. Radiographic visibility of periodontal ligament in mandibular third molars on both sides for males and females Abbreviations N – Number of participants, SD – Standard Deviation

Sex	Tooth	Stage	N	Mean	SD	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Males	38	0	19	18.55	2.11	16.11	17.91	18.25	18.50	26.05
		1	26	21.09	2.20	17.71	18.75	21.18	22.21	25.61
		2	61	21.07	2.68	16.46	19.24	20.65	22.79	27.37
		3	77	22.81	2.32	16.61	21.28	22.68	25.03	28.26
	48	0	19	18.52	2.12	16.11	17.78	18.25	18.55	26.05
		1	28	21.42	2.20	18.41	18.99	21.93	22.61	25.61
		2	54	21.11	2.79	16.46	19.24	20.48	23.04	27.37
		3	82	22.57	2.38	16.61	20.95	22.61	24.42	28.26
Females	38	0	51	18.86	1.99	15.76	17.45	18.80	19.78	26.70
		1	23	19.92	2.18	16.60	18.40	19.61	21.49	24.21
		2	74	21.74	2.53	16.91	20.04	21.19	23.16	28.08
		3	72	22.70	2.21	18.71	20.90	22.44	24.22	28.75
	48	0	50	18.77	1.95	15.76	17.45	18.73	19.54	26.70
		1	30	20.30	2.17	16.60	18.90	20.01	21.88	24.21
		2	75	21.67	2.4	16.91	20.21	21.19	22.98	28.08
		3	65	22.92	2.22	18.95	21.77	22.60	24.80	28.75

Utilizing Roberts method, the study discovered that the minimum age for categories RCW-A, RCW-B, and RCW-C was under 18 years of age for both sexes and assessed sides. This study included an additional category, RCW-U, which does not correspond to the original Roberts classification. Notably, the minimum age for RCW-U exceeded the 18 years of age threshold for males on the right side and females on the left side. Further details on mean age, minimum and maximum ages, and upper and lower quartiles for all Roberts method categories are provided in Table 5.

In the assessment of majority status, the root canal visibility of mandibular first, second, and third molars on both sides was evaluated for males and females. The analysis of the left side mandibular molars indicated an increasing probability of individuals being above 18 years of age with advancing stages. Stage 3 of visibility in all molars demonstrated a high likelihood of individuals having reached the age of majority in both sexes. Similarly, Stage 2 of the mandibular left second and third molars showed comparable precision in determining majority status. Detailed frequencies of individuals classified as having attained majority status based on left side mandibular molar visibility are presented in Table 6.

Table 5. Patterns of Root canal width visibility of mandibular first, second, and third molars on both sides and both sexes. N – Number of participants, SD – Standard Deviation, RCW – Root Canal Width, A, B, C, U stands for different types of RCW.

Sex	Side	Type	N	Mean	SD	Minimum	Lower quartile	Median	Upper quartile	Maximum
Males	Right	RCW-A	22	19.39	2.38	16.56	17.98	18.34	20.45	26.05
		RCW-B	40	19.47	1.65	16.11	18.57	19.49	20.55	23.05
		RCW-C	84	22.74	2.53	16.61	21.31	22.68	24.76	28.26
		RCW-U	37	22.35	2.24	18.06	20.81	22.03	24.36	26.13
	Left	RCW-A	20	19.06	1.91	16.56	18.00	18.26	19.75	22.79
		RCW-B	38	19.89	2.28	16.11	18.64	19.41	21.11	26.05
		RCW-C	81	22.76	2.51	16.61	21.40	22.68	24.76	28.26
		RCW-U	44	21.85	2.34	17.92	20.25	21.32	23.68	26.13
Females	Right	RCW-A	41	19.67	2.51	15.76	17.43	19.59	21.30	25.61
		RCW-B	56	19.71	1.95	16.02	18.45	19.42	20.86	23.86
		RCW-C	76	22.68	2.33	17.20	20.99	22.59	23.97	27.97
		RCW-U	47	21.90	2.72	16.57	20.12	21.20	23.19	28.75
	Left	RCW-A	51	19.30	2.18	15.76	17.43	19.14	20.85	24.74
		RCW-B	36	20.18	2.59	16.02	18.45	19.95	21.15	26.24
		RCW-C	78	22.37	2.50	17.20	20.64	22.56	23.86	27.97
		RCW-U	55	21.95	2.37	18.51	20.24	21.25	23.14	28.75

Table 6. Stage distribution according to majority status (< 18 years or ≥ 18 years) based on root canal visibility of mandibular first (36), second (37), and third molars (38) for both males and females on the left side Abbreviations n = number of participants, % indicates frequency of distribution

Root Canal Visibility	Status	36		37		38	
		Males n (%)	Females n (%)	Males n (%)	Females n (%)	Males n (%)	Females n (%)
Stage 0	< 18 years	-	-	-	0 (0)	5 (35.72)	11 (42.31)
	≥ 18 years	-	-	-	3 (100)	14 (73.68)	15 (57.69)
	Total	-	-	-	3 (100)	19 (100)	26 (100)
Stage 1	< 18 years	9 (16.67)	14 (19.44)	13 (10.66)	25 (17.86)	2 (7.69)	11 (44.00)
	≥ 18 years	45 (83.33)	58 (80.56)	109 (89.34)	115 (82.14)	24 (92.31)	14 (56.00)
	Total	54 (100)	72 (100)	122 (100)	140 (100)	26 (100)	25 (100)
Stage 2	< 18 years	4 (14.50)	13 (12.38)	2 (2.63)	1 (1.72)	8 (2.63)	3 (6.00)
	≥ 18 years	83 (85.50)	92 (87.62)	40 (97.37)	57 (97.37)	53 (97.37)	47 (94.00)
	Total	87 (100)	105 (100)	38 (100)	58 (100)	61 (100)	50 (100)
Stage 3	< 18 years	3 (7.14)	2 (4.65)	0 (0)	0 (0)	1 (1.30)	4 (3.36)
	≥ 18 years	39 (92.86)	41 (95.35)	11 (100)	19 (100)	76 (98.70)	115 (96.64)
	Total	42 (100)	43 (100)	11 (100)	19 (100)	77 (100)	119 (100)

In the corresponding analysis on the right side, the data revealed that for both sexes, the likelihood of an individual reaching the 18 years of age threshold was greater in stage 3 for all mandibular molars in females than in males. Notably, stage 2 of the mandibular first molar exhibited a higher frequency of correctly classifying individuals according to majority status. Interestingly, stage 1 of mandibular second and third molars demonstrated a superior frequency rate compared to stage 2 in terms of root canal visibility. The frequency of individuals achieving majority status with mandibular left first, second, and third molars is outlined in Table 7.

Table 7. Stage distribution according to majority status (< 18 years or ≥ 18 years) based on root canal visibility of mandibular first (46), second (47), and third molars (48) for both males and females on the right side Abbreviations n = number of participants, % indicates the frequency of distribution

Root Canal Visibility	Status	46		47		48	
		Males n (%)	Females n (%)	Males n (%)	Females n (%)	Males n (%)	Females n (%)
Stage 0	< 18 years	-	-	-	0 (0)	6 (31.58)	12 (42.86)
	≥ 18 years	-	-	-	2 (100)	13 (68.42)	16 (57.14)
	Total	-	-	-	2 (100)	19 (100)	28 (100)
Stage 1	< 18 years	7 (12.50)	15 (23.81)	13 (9.56)	28 (19.44)	1 (3.57)	11 (47.83)
	≥ 18 years	49 (87.50)	48 (76.19)	123 (90.44)	116 (80.56)	27 (96.43)	12 (52.17)
	Total	56 (100)	63 (100)	136 (100)	144 (100)	28 (100)	23 (100)
Stage 2	< 18 years	7 (6.00)	13 (11.21)	3 (11.17)	4 (6.90)	8 (11.17)	4 (7.27)
	≥ 18 years	88 (94.00)	103 (88.79)	33 (88.83)	54 (93.10)	46 (88.83)	51 (92.73)
	Total	100 (100)	116 (100)	403 (100)	58 (100)	54 (100)	55 (100)
Stage 3	< 18 years	2 (6.25)	1 (2.44)	1 (5.26)	0 (0)	1 (1.22)	2 (1.75)
	≥ 18 years	30 (93.75)	40 (97.56)	18 (94.74)	16 (100)	81 (98.78)	112 (98.25)
	Total	32 (100)	41 (100)	19 (100)	16 (100)	82 (100)	114 (100)

DISCUSSION

In medico-legal practice, accurate age estimation by forensic odontologists is crucial for the appropriate management of individuals in various legal contexts, particularly regarding juvenile justice proceedings. [23] This is due to the distinct legal ramifications associated with age thresholds such as 14, 16, 18, and 21 years. [23] Beyond the legal sphere, age determination also plays a pivotal role in establishing eligibility for educational opportunities, employment, marriage, and social benefits (e.g. pension), highlighting its broader societal significance. [16]

In forensic evaluations requiring age verification, particularly for legal proceedings involving minors, accurate assessment of the 18 years of age threshold is crucial. While third molar development has been proposed as a method for age estimation, its reliability has been challenged due to documented cases of early mineralisation in individuals below 18 years old. [24-26] Olze et al., [27] addressed this challenge by proposing a novel method for assessing pulp visibility (secondary dentine formation) in orthopantomograms, aiming to enhance forensic certainty in dental age estimation.

The progressive deposition of secondary dentine throughout life diminishes the pulp chambers and root canals, readily observable on dental radiographs. [28-29] Inspired by this age-related phenomenon, Olze et al. [18, 22] devised two novel dental techniques to evaluate the radiographic visibility of root pulp and periodontal ligament in lower third molars for forensic age estimation. Due to inherent variations in third molar development, employing a combination of these methods yielded superior age-predictive accuracy

compared to single-method approaches. [30] Considering the documented frequency of missing mandibular third molars (up to 28% [31], 46-60% [3]), which could skew results, this study adopted a differentiated assessment approach. Root pulp visibility was assessed bilaterally in all mandibular molars, while PDL visibility was assessed bilaterally only in mandibular third molars.

Mandibular first molars were pioneered for age estimation by Balla et al., [14] to evaluate their radiographic visibility as maturity markers. They observed that the minimum age was approximately 14 years in males and females for stages 0 and 1, irrespective of the side assessed. Interestingly, the minimum age was over 18 years for stage 3 in males compared to females (16 years approximately). Mantapuri et al., [32] further supported this age range, with stages 0 and 1 reported in the age of 12 years in both males and females. This study, however, found a complete absence of stage 0 in both sexes. Notably, the minimum age for stages 1, 2, and 3 was higher in both sexes compared to previous reports, with minimum age reaching 20, 21, and 22 years respectively.

Balla et al. [13] investigated the root pulp visibility in mandibular second molars as an age indicator. Their findings revealed that at 14 years of age, stage 0 was noted, regardless of sex. Stage 1 was observed at a minimum age of 14.74 years, whereas stage 2 was observed above 18 years in males and below 18 years in females. In this study, a near-identical minimum age of approximately 21 years was noted for both stages 1 and 2 in males and females. While stage 0 was present in a limited number of females, it was absent in males. Notably, stage 3, absent in the previous report, presented with a minimum age of approximately 23 years in both sexes, in the current study.

Previous investigations by many researchers [2-3, 18-19, 33-35] have explored the relationship between root pulp visibility (RPV) in mandibular third molars and chronological age in males. These studies reported minimum ages for RPV stages 0-3 ranging from 16.3 to 18.2 years for stage 0, 16.5 to 21.0 years for stage 1, 18.1 to 25.3 years for stage 2, and 19.1 to 26.5 years for stage 3. Similarly, in these studies, the minimum ages for RPV stages 0-3 ranged from 16.3 to 18.8 years for stage 0, 16.1 to 21.6 years for stage 1, 18.1 to 23.4 years for stage 2, and 21.2 to 27.7 years for stage 3. Consistent with these findings, this study identified minimum ages of approximately 16 years for RPV stages 0, 2, and 3 in male participants, and minimum ages of 15 years for stage 0, and 16 years for stage 1 and 2 in female participants, respectively. Also, stage 3 of PLV in females was found above the 18 years of age threshold.

The mesio-distal width of mandibular molars on the left side, as assessed by Roberts et al., [20] serves as a potential human biological growth marker for identifying individuals exceeding the 18 years of age threshold. Their study identified three distinct patterns (RCW-A, RCW-B, and RCW-C) with a minimum age over 18 years for categories RCW-C, irrespective of sex. These findings were corroborated by Davidson et al., [36] who further introduced a new category (RCW-U) for teeth not readily classified in the initial types. The results of this study were in concordance with previous studies, with the minimum age for all patterns (RCW A, B, and C) was 16 years in males for both sides, whereas 15 years was noted for RCW-A, 16 years for RCW-B, and 17 years for RCW-C in females for both sides.

This study evaluated the predictive accuracy of mandibular molar pulp visibility stages for age classification (<18 years vs. ≥18 years). Stage 2 was identified as the potential threshold in mandibular first molars, with previous reports [14] indicating 20.4% and 16.9% false positive rates for males and females, respectively. Similar rates of false positives were noted in this study (10.25% for males and 11.8% for females), suggesting that stage 2 may be a reliable indicator of age within acceptable error margins.

In a study by Balla et al., [13] the mandibular second molar demonstrated high accuracy in sex classification, with 100% for males and 79.2% for females. Notably in this study, left side molars displayed superior predictive power compared to right side counterparts, with 97% accuracy for both sexes on the left, compared to 88% and 93% for males and females on the right, respectively.

This study was limited by an imbalance in group sizes between males and females, and the exclusion of individuals with incomplete root formation, which resulted in a narrower age range (16-25 years) compared to the potential population (up to 40 years). Also, the participant cohort was above 16 years questioning the credibility of the assessment in the age group below it for assessment of mandibular first and second molars. Predictive classification using ROC curve analysis could not be carried out due

to improper distribution of samples in various age groups and the stages of pulp visibility seen. The prospective design was necessitated by radiographic regulations, but future retrospective studies with a wider age range and consideration of factors like ethnicity and dietary habits could provide more generalised findings on the radiographic visibility of root pulp and periodontal ligament in mandibular molars.

CONCLUSION

The present study demonstrates a significant positive correlation between increasing stages of pulp and periodontal ligament visibility in mandibular molars and the attainment of legal adulthood (18 years of age). This correlation holds irrespective of the specific tooth assessed.

Key findings include:

- Mandibular first molars exhibiting stage 2 or 3 pulp visibility exhibit a high probability (> 90%) of belonging to individuals above 18 years of age.
- Stage 2 pulp visibility in mandibular second molars compared to stage 1 shows a significantly higher likelihood (> 94%) of exceeding the legal age threshold.
- Both root pulp and periodontal ligament visibility in mandibular third molars can be used for age estimation with increasing accuracy from stage 2 to 3.
- Application of Roberts method to assess mandibular molar root canal width reveals that individuals categorised as RCW-C have a substantially higher probability of being above 18 years old.

These findings suggest the potential of mandibular molar visibility and root canal morphology as reliable indicators of legal age in forensic and anthropological contexts.

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