Validation of radiographic visibility of root pulp in mandibular first, second and third molars in the prediction of 21 years in a sample of south Indian population: A digital panoramic radiographic study

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KEYWORDS

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ABSTRACT

This study examines the radiographic visibility of root pulp (RPV) in lower first, second and third molars to validate the completion of 21 years. RPV in all lower three molars of both sides was assessed using a sample of 930 orthopantomograms of individuals aged between 15 and 30. The scoring of RPV was done using the Olze et al. four-stage classification (Int J Legal Med 124(3):183–186, 2010). Cut-off values were determined for each molar using the receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC). The selected cut-off values were stage 3 for the first molar, stage 2 for the second molar and stage 1 for the third molar. For lower first molar, the AUC was 0.702, and the sensitivity, specificity and posttest probability (PTP) were 60.1%, 98.8% and 98.1% in males, and 64.5%, 99.1% and 98.6% in females. For lower second molar, the AUC was 0.828, and the sensitivity, specificity and PTP were 75.5%, 97% and 96.2% in males, and 74.4%, 96.3% and 95.3% in females. For the lower third molar, the AUC was 0.906; the sensitivity was 74.1% and 64.4% in males and females, while specificity and PTP were 100% in both sexes. The accuracy of predictions for the completion of 21 years was high. However, the greater percentage of false negatives and inapplicability of this method in one-third of lower-third molars have been recommended for using this method in conjunction with other dental or skeletal methods.

INTRODUCTION

An essential aspect of age assessment practice is the correct discrimination of the subjects according to the age threshold in question and reducing false categorisation. The increase in the proportion of individuals who were unable to or unwilling to disclose their actual age made it necessary for age assessments to provide justice. Besides legal issues and issues related to undocumented migrants, age estimation practices in adolescents and sub-adults also concern the field of competitive sports.[1, 2] In such instances, the completion of the 18th year and 21st year of life is validated of paramount importance. In India, the completion of the 21st year of life is essential for marriage in males.[3, 4]

The development and the completion of third molars is a subject of interest in forensic and medico-legal practice as it provides proof of attainment of legal ages 16 and 18. However, the evidence of completion of third molar development even before 18 years makes it difficult to validation of the completion of legal

age thresholds.[5] This necessitates alternative markers associated with normal ageing to predict the completion of the 21st year of life. In 2010, Olze et al.[6] described a four-stage classification based on the root pulp visibility in lower third molars. It studies the changes in the appearance of root canals of lower third molars that could relate to secondary dentin deposition and appositional bone growth.[7] Later, several researchers tested this method in different populations and concluded that this method could be reliable, reproducible, and helpful in assessing ages over 18 and 21 years.[7-14] Few researchers tested the applicability of this method in first and second molars and recommended the use of this method for predicting the age over 14, 16 and 18 years in the absence of third molars.[15-18]

To date, researchers have only studied the applicability of this method in lower third molars to predict the attainment of the legal age of 21 years.[11] To the best of our knowledge, no studies were available in the literature studying this pattern in lower first and second molars to predict 21 years. Therefore, the present paper explores the potential application of root pulp visibility in mandibular first, second and third molars to determine whether or not a subject in question is under or over the 21-year threshold.

MATERIALS AND METHODS

Sample collection and selection criteria

A total of 930 OPGs (465 males and 465 females) from adolescents, young adults, and adults of South Indian origin aged between 16 and 30 years were collected. All the OPGs were obtained retrospectively from the archives of private dental clinics in 2017 and 2020. The inclusion criteria were radiographic images of good diagnostic quality with at least one mandibular first, second, and third molars. The most typical reasons for excluding radiographs were molars with caries, restorations, evidence of endodontic treatment, and dental anomalies. A few more reasons are one-rooted and molar teeth with root foreshortening due to perspective.

After meeting the selection criteria, each OPG was given a unique identification number (UIN). Details of sex, date of birth, and the date on which the radiograph was taken were entered against each UIN. The chronological age of each individual is obtained after calculating the difference between the date of birth and the date of exposure.

Method

In each OPG, the lower first, second, and third molars from both sides were categorised using the Olze et al. four-stage classification of root pulp visibility (Figure 1).[6] In stage 0, the lumen of the root canals is visible up to the apex. In stage 1, the lumen of one root canal is discernible up to the root apex; in stage 2, two root canals with incompletely visible lumen to the apex or one canal might be virtually not visible in entire length; and in stage 3, the lumen of two root canals is almost not visible in entire length.

All the OPGs were analysed and graded by a forensic odontologist with more than eight years of experience in forensic age estimation. To study the intraobserver variability, the researchers randomly selected 90 OPGs to re-evaluate. The minimum time interval between both examinations was one month. And to explore the interobserver variability, the same number of OPGs was evaluated by another investigator.

Statistical analysis

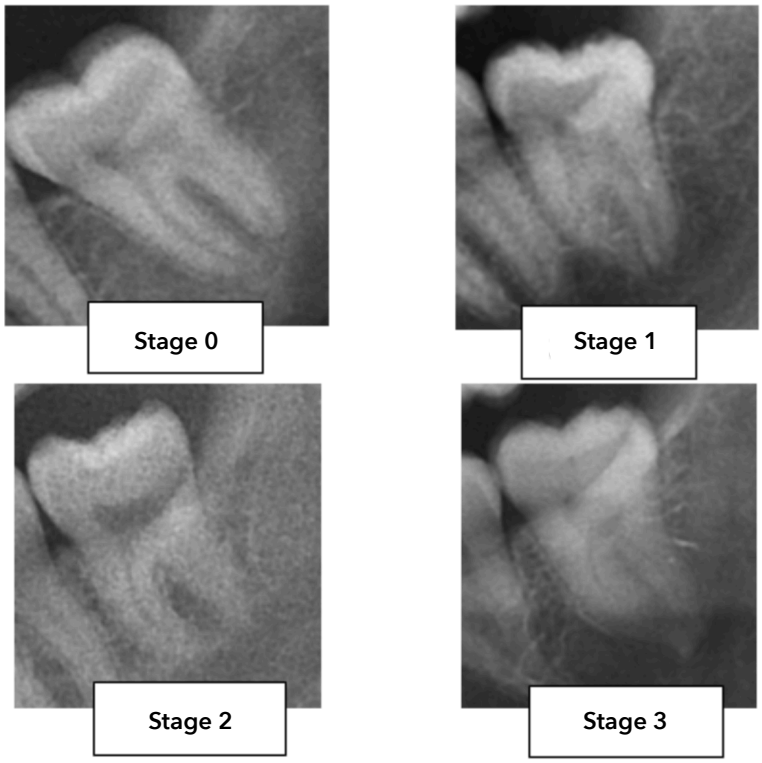
IBM SPSS Statistics for Windows v20 (IBM Corp., Armonk, NY) was used to perform statistical analysis, and the level of significance was set at 5% ($p < 0.05$). Inter- and intra-observer agreements were calculated using Cohen's kappa test. Descriptive statistics, including mean, standard deviation, minimum age, maximum age, and median, were performed for each stage in all lower molars for both sexes. Chronological age was recorded as a binary variable with a cut-off at 21 years old, i.e., "0" for subjects under 21 years and "1" for those above 21 years. Chi-square tests were used to test the relationship between chronological age and stage attainment. Receiver operating characteristic (ROC) curve analysis was performed, and we sought optimal grade cut-offs to classify subjects into particular categories. As sex is a possible confounding variable, we performed separate ROC analyses for both sexes.

The performance of the cut-off values, i.e., stages of root pulp visibility of lower three molars, were evaluated by calculating the areas under the ROC curve (AUC), sensitivity, specificity, and likelihood ratios (LR+ and LR-). AUC is a measure of the accuracy of a quantitative diagnostic test. It represents the overall performance of the stages of root pulp visibility

in discriminating subjects under or over the 21-year threshold. The sensitivity indicates the probability that cases will be correctly diagnosed as being 21 or older; specificity suggests the possibility that controls will be correctly interpreted as younger than 21. The likelihood ratio combines sensitivity and specificity into a single value that indicates how much the test result will reduce the uncertainty of a given diagnosis.[19, 20] For example, LR+ indicates how many true positives will be observed per false positive. Therefore, an LR+ of >1 suggests a positive result is likely correct. The greater the value, the higher the probability that the outcome is correct. On the other hand, LR– indicates how many false negatives will be observed per true negative. The closer the LR– value is to 0, the higher the discrimination potential of the cut-off value.

The root pulp visibility stages in the first, second, and third molars may help discriminate between individuals under or over the legal age of 21 years by the Bayes post-test probability. Using the census data of India (http://www.censusindia.gov.in/2011census/C- series/C-13.html), we assumed the possibility of the individual in question being 21 or older in the target population between 15 and 30 years is 0.50 in males and females.

Figure 1. Diagrammatic representation of Olze et al stages of root pulp visibility in mandibular molars



RESULTS

Sample studied and excluded OPGs

Table 1 displays the age and sex distribution of the overall sample. The mean age of the males and females was 21.65 ± 4.33 and 21.64 ± 4.36 years, respectively.

Table 2 lists the reasons for excluding OPGs or third molars from evaluation. Approximately one-third of lower third molars (right and left) in females and 28% in males were not eligible for evaluation. The most common reason for their exclusion is the developing third molars with open apices; missing third molars (below 5% in both sexes) is another reason. Most developing third molars were seen below 20 years of age.

Intraobserver and interobserver agreements

Cohen kappa statistics revealed that the intraobserver agreement was 0.845 ($p < 0.001$) with a 95% confidence interval (CI) of (0.731, 0.946), indicating almost perfect agreement. On the other hand, the interobserver agreement was 0.762 ($p<0.001$) with a 95% confidence interval (CI) of (0.628, 0.891), indicating a substantial agreement.

Data analysis

Table 3 shows the results of descriptive statistics, i.e., mean, standard deviation, minimum age, maximum age, and median for each stage of root pulp visibility in lower first, second and third molars (right and left) in both sexes. Table 4 displays the proportion of subjects under and over the 21-year-old thresholds for each stage of root pulp visibility in both sexes.

Table 5 shows the performance of root pulp visibility stages at the optimal cut-offs, i.e., stage 3 in the lower first molar, stage 2 in the lower second molar, and stage 1 in the lower third molar for indicating the age above 21 years. ROC curve analysis showed that stage 3 of root pulp visibility in the lower first molar exhibited moderate discriminatory capacity (AUC, 0.702; 95% CI, 0.668- 0.736) and stage 2 in the lower second molar (AUC, 0.828; 95% CI, 0.801- 0.856) and stage 1 in the lower third molar (AUC, 0.906; 95% CI, 0.884- 0.929) has exhibited high discriminatory capacity (Figures 2 to 4).

Table 1. Age and sex distribution of the total sample

Age groups	Males	Females	Total
15- 15.9 years	40	40	80
16- 16.9 years	40	40	80
17- 17.9 years	40	40	80
18- 18.9 years	40	40	80
19- 19.9 years	40	40	80
20- 20.9 years	30	30	60
21- 21.9 years	30	30	60
22- 22.9 years	30	30	60
23- 23.9 years	25	25	50
24- 24.9 years	25	25	50
25- 25.9 years	25	25	50
26- 26.9 years	25	25	50
27- 27.9 years	25	25	50
28- 28.9 years	25	25	50
29- 29.9 years	25	25	50
Total	**465**	**465**	**930**

Table 2. Age and sex distribution of the total sample

Reason for exclusion	Tooth 38		Tooth 48	
	Males	Females	Males	Females
Developing	117 (25.1)	135 (29)	118 (25.3)	134 (28.8)
Missing	14 (3.1)	20 (4.3)	13 (2.8)	16 (3.4)
Total	131 (28.1)	155 (33.3)	131 (28.1)	150 (32.2)

Tooth 38, Lower left third molar; Tooth 48, Lower right third molar

Table 3. Descriptive statistics of chronological age according to sex and RPV stages in lower first, second and third molars of both sides

Teeth	Stage	Males						Stage	Females					
		n	Mean	SD	Min	Max	Median		n	Mean	SD	Min	Max	Median
36	0	45	16.69	1.11	15.10	18.98	16.59	0	42	16.25	0.83	15.05	18.43	16.25
	1	117	17.83	1.85	15.02	21.91	17.62	1	125	17.96	1.92	15.01	21.99	17.89
	2	219	22.71	3.41	15.50	29.91	22.31	2	188	22.05	2.93	16.23	19.22	22.12
	3	84	26.87	2.14	15.06	29.92	26.85	3	110	27.19	1.98	17.51	29.96	27.47
46	0	40	16.56	0.99	15.10	18.98	16.34	0	44	16.29	0.85	15.05	18.43	16.26
	1	123	17.82	1.83	15.02	21.91	17.62	1	125	17.99	1.96	15.01	22.71	17.89
	2	224	22.79	3.36	15.50	29.91	22.39	2	196	22.26	2.97	16.23	29.22	22.34
	3	78	27.02	2.10	15.06	29.92	26.99	3	100	27.34	1.95	17.51	29.96	27.55
37	0	163	17.53	1.82	15.02	22.59	17.33	0	158	17.43	1.86	15.01	22.45	17.08
	1	135	21.01	2.51	15.06	26.91	20.76	1	143	21.08	2.67	16.15	26.83	20.55
	2	141	25.80	2.53	20.03	29.91	26.09	2	130	25.63	2.51	20.28	29.94	25.65
	3	26	28.23	1.26	25.82	29.92	28.28	3	34	28.33	0.99	25.59	29.96	28.17
47	0	160	17.51	1.84	15.02	22.59	17.20	0	158	17.46	1.90	15.01	22.45	17.08
	1	142	21.02	2.52	15.06	26.91	20.71	1	148	21.17	2.81	16.15	29.94	20.56
	2	154	26.11	2.51	20.03	29.92	26.31	2	134	25.82	2.42	20.28	29.90	25.91
	3	09	28.66	0.53	27.88	29.43	28.66	3	25	28.50	1.04	25.59	29.96	28.31
38	0	143	20.08	1.99	15.06	26.50	19.79	0	136	20.55	2.35	17.05	26.81	20.23
	1	188	25.81	2.51	21.08	29.92	26.05	1	174	25.96	2.52	21.02	29.96	26.25
	2	03	29.24	0.15	29.14	29.43	29.17	2	--	--	--	--	--	--
	3	--	--	--	--	--	--	3	--	--	--	--	--	--
48	0	168	20.63	2.33	15.06	27.46	20.09	0	170	21.09	2.46	17.05	26.83	20.61
	1	163	26.09	2.48	21.08	29.92	26.36	1	144	26.51	2.33	21.02	29.96	26.93
	2	03	29.24	0.15	29.14	29.43	29.17	2	01	--	--	--	--	--
	3	--	--	--	--	--	--	3	--	--	--	--	--	--

n, number; SD, Standard deviation; Min, Minimum age; Max, Maximum age.

Table 4. Proportion of subjects under and over 21 years according to the RPV stages for all lower three molars in both sexes

Tooth	Age category	Males				Females			
		Stage 0 n (%)	Stage 1 n (%)	Stage 2 n (%)	Stage 3 n (%)	Stage 0 n (%)	Stage 1 n (%)	Stage 2 n (%)	Stage 3 n (%)
36	<21 years	45 (9.6)	110 (23.6)	74 (16)	01 (0.2)	42 (9)	116 (25)	71 (15.2)	01 (0.2)
	≥21 years	0 (0)	07 (1.5)	145 (31.1)	83 (17.8)	0 (0)	09 (1.9)	117 (25.1)	109 (23.4)
37	<21 years	152 (32.6)	73 (15.6)	05 (1.07)	00 (0)	149 (32)	75 (16.1)	06 (1.3)	00 (0)
	≥21 years	11 (2.3)	62 (13.3)	136 (29.2)	26 (5.6)	09 (1.9)	68 (14.6)	124 (26.6)	34 (7.3)
38	<21 years	106 (22.8)	00 (0)	00 (0)	00 (0)	89 (19.1)	00 (0)	00 (0)	00 (0)
	≥21 years	37 (7.9)	188 (40.3)	03 (0.6)	00 (0)	47 (10.1)	174 (37.4)	00 (0)	00 (0)

Table 5. Performance measures of root pulp visibility (RPV) in lower first, second and third molars for discriminating legal age 21 years

Quantity	Tooth 36	Tooth 37	Tooth 38
Males			
Accuracy	67.1 (62.6- 71.3)	83.2 (79.5- 86.5)	88.9 (85.1- 92.1)
Sensitivity	60.1 (54.9- 65.06)	75.5 (70.2- 80.3)	74.1 (66.1- 81.1)
Specificity	98.8 (93.5- 99.9)	97.01 (93.1- 99.02)	100 (98.1- 100)
Positive LHR	50.49 (7.18- 354.87)	25.22 (10.61- 59.94)	--
Negative LHR	0.40 (0.36- 0.46)	0.25 (0.21- 0.31)	0.26 (0.2- 0.34)
PTP	98.1 (87.8- 99.7)	96.2 (91.4- 98.4)	100
Females			
Accuracy	72.7 (68.3- 76.7)	82.1 (78.3- 85.5)	84.4 (80.3- 88.6)
Sensitivity	64.5 (59.2- 69.5)	74.4 (69.1- 79.2)	65.4 (56.8- 73.3)
Specificity	99.09 (95.04- 99.9)	96.3 (92.2- 98.6)	100 (97.9- 100)
Positive LHR	70.96 (10.07- 500.04)	20.34 (9.25- 44.74)	--
Negative LHR	0.36 (0.31- 0.41)	0.27 (0.22- 0.32)	0.35 (0.27- 0.44)
PTP	98.6 (91- 99.8)	95.3 (90.2- 97.8)	100

LHR, Likelihood ratio; PTP, Posttest probability

Figure 2. ROC curve for stage 3 root pulp visibility in lower first molar (Tooth 36) for 21-year threshold

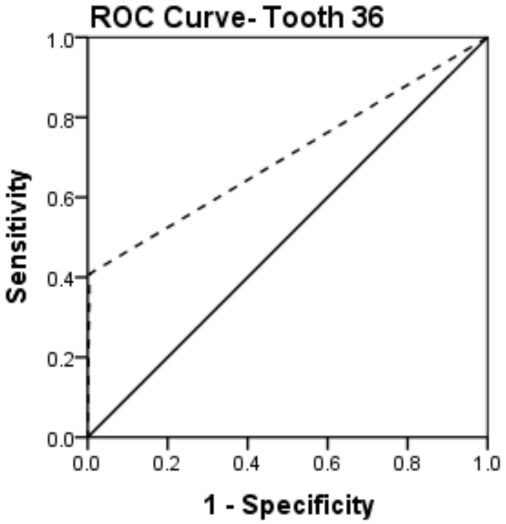


Figure 3. ROC curve for stage 2 root pulp visibility in lower second molar (Tooth 37) for 21-year threshold

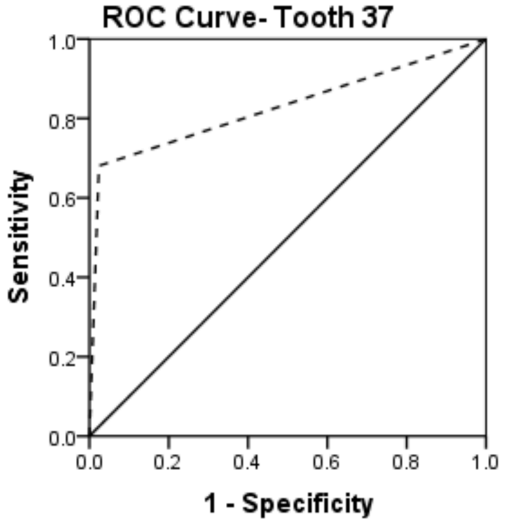
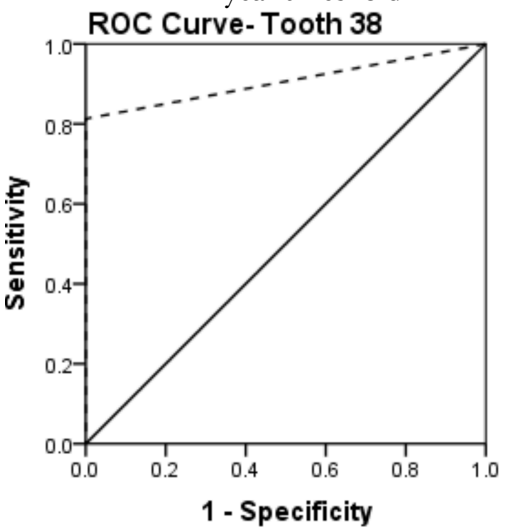


Figure 4. ROC curve for stage 3 root pulp visibility in lower third molar (Tooth 38) for 21-year threshold



DISCUSSION

It is essential to reduce false categorisation in age assessment practice. According to Akkaya N et al.,[11], a perfect "gold standard" age estimation method does not exist as all methods are associated with some errors. Furthermore, using techniques in conjunction with other methods is recommended, mainly while categorising individuals as above or below the age threshold of medicolegal importance. For example, a four-stage classification of root pulp visibility in lower third molars by Olze et al. [6] seems to be accurate in discriminating individuals older than 18 and 21 years. However, Al Qattan et al. [7] have stated multiple reasons (developing third molars, third molars with single roots, buccolingually tilted molars, missing teeth, positional or morphological anomalies, etc.), suggesting that it is not a suitable method. Therefore, it is essential to test this parameter, i.e., root pulp visibility in alternative teeth in the absence of third molars, to predict the legal age of 21 years. Therefore, the present investigation assessed the root pulp visibility in lower first, second, and third molars for the discrimination of individuals over 21 years.

Our study findings confirmed that repeatability (intraobserver differences) was almost perfect, while the reproducibility (interobserver differences) was substantial. These substantial findings could be related to the measurement of pulp visibility in an ordinal manner with four possible scores. One factor that could influence reproducibility is subjectivity which could result in observer errors. And, also the resolution of the radiographic images could affect the display of anatomical details. Therefore, observer training and calibration are essential.

Excluded OPGs

The present investigation selected OPGs based on a strict inclusion criterion, especially for lower first and second molars. Therefore, radiographic images with tilted molars, positional anomalies, and images that challenge the analysis of the molars due to superimposition or perspective factors were excluded beforehand. On the other hand, we have included OPGs with developing molars with incomplete roots and missing teeth for lower third molars. It shows how many samples were excluded from analysis and the percentage

of third molars the method could predict age above 21 years.
Our results showed that approximately one-third of the OPGs in females (tooth 38, 33.3% and tooth 48, 32.2%) and 28.1% OPGs (tooth 38 & 48) in male subjects were excluded from analysis due to developing third molars. Around 3 to 5% of the excluded sample in both sexes were due to missing teeth (developmentally missing or extracted).

Data analysis- Third molars
Few researchers have studied the applicability of this method in discriminating subjects over 21 years.[6, 9- 12] In the original study by Olze et al. [6], the authors stated that if stage 1 was observed in the lower third molar of the examined individual, then he/ she is most probably 21 years of age, and the presence of stages 2 and 3 can safely put them above 21 years. Guo et al. [12], in their study among Northern Chinese subjects, found that individuals who were found to be in Stages 2 and 3 were at least older than 21 years. Timme et al. [10] reported very similar values. In another study by Gok et al., [21] individuals under stage 3 of root pulp visibility are over 21.
Similarly, Perez- Mongiovi et al. [9] also found stage 3 a helpful marker in Portuguese females. However, differences were noticed when our study findings were compared with others. In the present study, the earliest appearance (minimum age) of stage 1 was 21.08 years in males and 21.02 years in females. Very few male subjects (n= 3) were graded with stage 2 root pulp visibility with a minimum age of 29.14 years. None of them was graded with stage 3 in the studied age range. The possible explanation for the differences among studies could be research design, statistical approaches, sample age ranges, population differences, and inter-observer variations.
Based on our findings in lower third molars, when stage 1 root pulp visibility was observed in the studied subjects, they were at least above 21 years. However, when tested as a cut-off value for indicating age above 21, it resulted in a sensitivity of 74.1% and 65.4% in males and females, specificity, and posttest probability of 100% in both sexes. These lower sensitivity values indicate that one in four males and one in five females older than 21 could be wrongly identified as subjects below 21 years, resulting in false negatives.

Data analysis- First and second molars
To the best of our knowledge, this is the first Indian study to verify the stages of root pulp visibility in lower first and second molars to predict the legal age of 21 years. In our research, the earliest observation of stage 0 in males and females for lower first molars were 15.1 and 15.05 years, 15.02 and 15.01 years for stage 1, 15.50 and 16.23 years for stage 2, and 15.06 and 17.51 years for stage 3, respectively. When stage 3 of root pulp visibility in lower first molars was used as a cut-off value to predict the completion of 21 years, it resulted in a sensitivity of 60.1% and 64.5% in males and females, specificity of 98.8% and 99.1%, and posttest probability of 98.1% and 98.6% in both sexes.
The earliest observation of Stage 0 for lower second molars was observed at 15.02 in males and 15.01 in females, stage 1 at 15.06 and 16.15, stage 2 at 20.03 and 20.28, and Stage 3 at 25.82 and 25.59 years, respectively. When stage 2 of root pulp visibility in lower second molars was used as a cut-off value to predict the completion of 21 years, it resulted in a sensitivity of 75.5% and 74.4% in males and females, specificity of 97.01% and 96.3%, and posttest probability of 96.2% and 96.3% in both sexes.

False categorisation/ errors
In forensic age estimation, two types of errors can occur, i.e., ethically unacceptable errors/ false positives (Type I) and technically unacceptable errors/ false negatives (Type II).[22] Although all errors should be kept to a minimum in forensic age estimation, it is essential that type I errors must be eliminated.
In the present study, a more significant percentage of type II errors was observed when stages of root pulp visibility in lower first, second and third molars were used as age markers for ages over 21 years. When stage 3 root pulp visibility in the lower first molar was used, it resulted in less than 1% false positives and 59% false negatives. Stage 2 root pulp visibility in the lower second molar resulted in 2.4% false positives and 32% false negatives. On the other hand, stage 1 root pulp visibility in the lower third molar resulted in zero false positives and 18.7% false negatives.

Strengths, limitations and future considerations
One of the strengths of the present study is the equal distribution of samples (with matching

males and females samples) among the age groups studied. Secondly, our study findings have provided an alternative parameter for determining the completion of the 21st year of life without third molars. These were crucial for predicting legal ages 18 and 21. Our study has some limitations. Mainly, the presence of a higher percentage of developing third molars or missing third molars is excluded from evaluation. Further studies are warranted to improve the interobserver differences using continuous markers, such as root pulp area, which may better the predictive performance. Future studies should investigate the influence of ethnicity and dietary habits on root pulp visibility.

CONCLUSION

Based on the study findings, the following conclusions can be made:

1. Subjects graded with stage 3 root pulp visibility in lower first molars were at least older than 21.
2. Subjects graded with stage 2 and 3 root pulp visibility in lower second molars were at least older than 21 years of age.
3. Subjects graded with stage 1, 2, and 3 root pulp visibility in lower third molars were at least older than 21 years of age.

Therefore, all the lower three molars have resulted in better specificity and posttest probability values (>90%), indicating that they could be reliable in forensic age estimation. However, it is advised to proceed with caution. They should be used in conjunction with other age estimation methods owing to the more significant percentage of false negatives with first and second molars and the inapplicability of this method in approximately one-third of the sample in lower third molars.

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