Testing the maturation and the radiographic visibility of the root pulp of mandibular third molars for predicting 21 years. A digital panoramic radiographic study in emerging adults of south Indian origin

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KEYWORDS

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ABSTRACT

Prediction of the attainment of legal age thresholds, especially in children and young adults, is a common task in medico-legal practice. In many countries, 21 years has medico-legal importance. In the present study, we assessed and compared the accuracy of the third molar maturity index (I3M) and the stages of radiographic visibility of the root pulp (RPV) in predicting the age threshold of 21 years. A sample of 910 digital panoramic radiographs (455 males and 455 females) of adolescents and young adults aged between 16 and 30 of south Indian origin were evaluated. The authors examined the performance of different I3M cut-off values and RPV stages. I3M cut-off value of 0.02 has resulted in better discrimination with an accuracy of 76.92% and 80.44%, specificity of 48.28% and 56.16% in males and females, a sensitivity of 100%, and post-test probability of 65.9% in both sexes. The accuracy and sensitivity of RPV stage 2 were 84.76% and 84.55%, 78.17%, and 78.97% in males and females, while the specificity and post-test probability were 100% in both sexes.

In conclusion, the I3M method resulted in a more significant percentage of false positives and cannot be used to state the attainment of 21 years. However, the presence of RPV stage 2 could say that the subject had already attained the age of 21 years. Further studies are warranted to address the usefulness of these methods.

INTRODUCTION

Assessment of the biological age of a living subject is a common practice in medico-legal, civil and social issues.[1] It becomes challenging for forensic experts when authorities want scientific proof of whether the examined subject is under or over the cut-off age of medico-legal importance. In India, as in many countries, it is required to provide proof of being under or over the legally defined age limits, especially for children who were part of criminal and medico-legal investigations. The applicable age limits in India for various legal issues range between 14 and 21 years of age (Table 1).[2] Lately, there has been increasing concern in India about raising the legal age of marriage for girls from 18 to 21 years.[3, 4] This highlights the need for age estimation methods to predict the legal age threshold of 21 years.

Choosing the parameters suitable for predicting the legal age of interest is essential in age estimation practice. According to the literature, teeth and their maturation are more reliable for

age assessment than skeletal parameters due to lower variability in the former than in the latter.[5] In addition, the calcification rate of the teeth is more controlled by genetic than environmental factors.[6] Since the development of the third molar extends into the early twenties, it has a unique advantage over other teeth and has become a subject of interest in forensic age assessments. The third molar maturity index (I3M) method, introduced by Cameriere et al. (2008)[7] for predicting the legal age of 18 years, has gained much popularity in forensic age estimation.[8] Several authors have applied the I3M method to predict other legal age thresholds, such as 14[9, 10,] and 16 years.[11]

Olze et al. [12] recognized the importance of an alternative dental method that can be applied to the third molars after root completion. Therefore, they introduced a stage classification that examined the radiographic visibility of the third molars' root pulp (RPV) for age estimation purposes. They concluded that this method could help predict 18 and 21 years of age. Researchers tested this method in other populations to predict 18 years,[13, 14], 21 years[15, 16,] and both 18 and 21 years together.[17- 19] In 2021, Pyata et al.[20] compared the accuracy of four dental age estimation methods to predict the legal age threshold of 18 years and concluded that radiographic visibility of root pulp in lower third molars is not a reliable tool due to the more significant percentage of third molars with incomplete mineralization in younger age groups. Alternatively, SB Balla et al.[21] and Suvarna et al.[22] studied this stage classification in mandibular first and second molars and proved its usefulness in estimating 18 years of age.

To the best of our knowledge, no study was available in the scientific literature to predict the legal age threshold of 21 years in emerging adults of south Indian origin. Therefore, in the present investigation, we aim to predict the attainment of the legal age threshold of 21 years using Cameriere's third molar maturity index (I3M). We also intend to study the radiographic visibility of the root pulp of the mandibular third molars**.** Additionally, we want to determine the accuracy of both methods.

Table 1. Legal area, legally relevant age limits, and legal issues in India

Legal area	Age limit (In years)	Legal issue (s)
Article 24 of Indian constitution	14 years	A child below 14 years shall not be employed to work in any factory or mine or engaged in other hazardous employment (Child labour)
Article 24 of Indian constitution	16- 18 years	Addresses children in conflict with law and children in need of care and protection.
Article 24 of Indian constitution	16 years	Sexual intercourse with a woman even with her consent is rape, if she was below 16 years of age
Article 24 of Indian constitution	18 years	Age of majority
Article 24 of Indian constitution		A person who is below 18 years of age is a minor
Article 24 of Indian constitution		Applicability of Criminal law, Capacity for actions and processes
Article 24 of Indian constitution	21 years	The legal age for marriage for a male

I.P.C; Indian Penal Code

MATERIAL AND METHODS

Sample

We evaluated digital panoramic radiographs of 910 adolescents and young adults (455 males and 455 females) of south Indian origin, ranging from 16 to 30 years (Table 2). Radiographs of the subjects who attended the private dental clinics that were pre-treatment in nature were collected retrospectively. Patient details such as the age, sex, date of birth, and date of exposure, i.e., the date on which the radiograph was taken, were recorded separately in an excel file. Each radiograph is assigned an identification code that matches the patient's details. It allows a blinded approach, i.e., the examiner is unaware of the patient information to avoid bias during the analysis. Radiographs were retrieved as digitalized images from the database and then stored in digital format (JPEG). The examiners use ImageJ computer software (version 1.48, National Institute of Health, USA) to perform an analysis of the radiographs. It allows them to use "magnify" and "ruler" tools for measurements. Radiographs showing the presence of at least one mandibular third molar with no evident bone pathology will be included. Radiographs of unknown age, sex, those with missing lower third molars on both sides were excluded. Radiographs that were of poor quality or distorted were also excluded.

Methodology

Third molar maturity (I3M) index

The I3M index of each mandibular third molar was studied using the method of Cameriere et al.[7] I3M is evaluated as the sum of the distances between the inner sides of the two open apices (X+Y) divided by the tooth length (Z) (Figure 1). A score of "0" is allocated when the development of the third molar is complete**.**

Radiographic visibility of the root pulp

The prediction of the legal age threshold of 21 years was also determined using the Olze et al. stages of RPV[12], which include stages 0 to 3. Stage "0" includes mandibular third molars with root canals visible to the apex. In contrast, stage "3" has mandibular third molars, with the lumen of two root canals virtually invisible in total length (Figure 2).

All the measurements, i.e., I3M measurements and RPV stage allocation, were performed by a single examiner (SBB), an experienced forensic odontologist. Later, SBB and the fourth author analyzed 100 randomly selected OPGs 6 weeks after the first analysis.

Table 2. Distribution of the total sample according to sex and age, numbers in parenthesis represent samples with open apices of the left third mandibular molars

Age group	Males	Females	Total
16- 16.99	40 (40.8)	40 (35.1)	80
17- 17.99	40 (32.7)	40 (30.7)	80
18- 18.99	40 (15.3)	40 (15.8)	80
19- 19.99	40 (8.2)	40 (17.5)	80
20- 20.99	43 (3.1)	43 (0.9)	86
21- 21.99	37 (0)	36 (0.9)	73
22- 22.99	40 (0)	40 (0)	80
23- 23.99	25 (0)	25 (0)	50
24- 24.99	25 (0)	26 (0)	51
25- 25.99	25 (0)	25 (0)	50
26- 26.99	25 (0)	25 (0)	50
27- 27.99	25 (0)	25 (0)	50
28- 28.99	25 (0)	25 (0)	50
29- 29.99	25 (0)	25 (0)	50
Total	455	455	910

Figure 1. Measurements of third molar maturity index. I3M is evaluated as the sum of the distances between the inner sides of the two open apices (X+Y) divided by tooth length (Z)

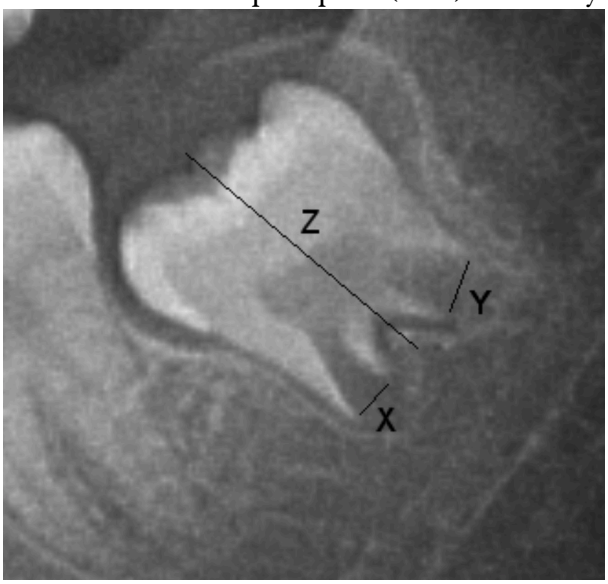
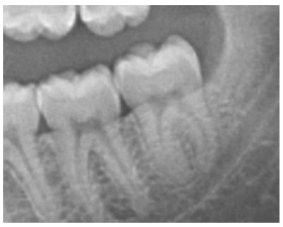
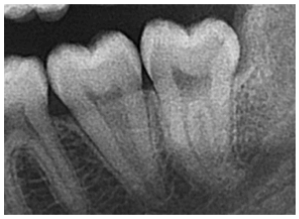
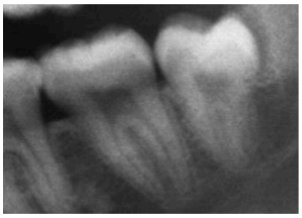


Figure 2. Images of the stages of radiographic visibility of the root pulp in mandibular third molars

Pulp visualization stages	Original Drawings of Olze et al.	Example X- rays for different stages in lower third molars in study population
STAGE 0		
STAGE 1		
STAGE 2		
STAGE 3		

Statistical analysis

Data analysis was performed using SPSS statistics v.20.0 for windows (IBM; Armonk; New York, USA). The significance level for the analysis was set at 5%. Intra- and inter-examiner agreement for both methods were calculated using the intra-class correlation coefficient (ICC) method. Four different widely used precision estimates were calculated: the technical error of measurement (TEM), the relative technical error of measurement (rTEM), and the coefficient of reliability (R) to study intra- and inter-examiner precision.

Descriptive statistics were performed. To test the performance of specific cut-off values of I3M and RPV stage, the results were summarized in a table consisting of 2x2 contingency tables. The details of the diagnostic performance indicators from a single 2x2 contingency table were determined and displayed in Table 3.

The post-test probability (p) of being 21 years of age or older can help to discriminate between those individuals who are 21 and over and those under 21. According to Bayes theorem, the post-test probability may be written as:

$$p = \frac{Se \times p0}{Se \times p0 + (1 - Sp) \times (1 - p0)}$$

where p is post-test probability and p_o is the probability that the subject in question is ≥ 21 years, given that they are aged between 16 and 30 years, representing the target population. Probability p_0 was calculated as the proportion of subjects between 21 and 30 years of age who live in Andhra Pradesh and Telangana and those between 16 and 30 years, which was evaluated from data from the same web source. This value ($p0$) was considered to be 0.50 for both males and females according to the demographic data from the 2011 census (http://www.censusindia.gov.in/2011census/C- series/C-13.html).

Table 3. Diagnostic performance indicators used in the comparative analysis

True positive	The number of participants who have I3M< 0.02 who are 21 years and older (OR) the number of participants who have RPV stage 2 or higher who are 21 years and older.
False positive	The number of participants who have I3M< 0.02 who are younger than 21 years (OR) the number of participants who have RPV stage 2 or higher who are younger than 21 years.
True negative	The number of participants who have I3M≥ 0.02 who are younger than 21 years (OR) the number of participants who have RPV stage 1 or lower who are younger than 21 years.
False negative	The number of participants who have I3M≥ 0.02 who are 21 years and older (OR) the number of participants who have RPV stage 1 or lower who are 21 years and older.
Accuracy	Proportion of correctly identified participants
Sensitivity	The proportion of the participants 21 years and older who had I3M or the RPV stage < cut-off
Specificity	The proportion of the participants younger than 21 years who had I3M or the RPV stage ≥ cut-off
Positive predictive value (PPV)	Tests that look at out of the positive tests of how many were truly positive and correctly classified
Negative predictive value (NPV)	Tests that look at out of the negative tests of how many were truly negative and correctly classified
Likelihood Ratio of the positive test (LR+)	The probability that an individual 21 and older selected positive for the tested age 21years (true positive) divided by the probability that an individual younger than 21 selected positive for the tested age 21 years (false positive).
Likelihood Ratio of the negative test (LR-)	The probability that an individual 21 and older selected negative for the tested age 21years (false negative) divided by the probability that an individual younger than 21 selected negative for the tested age 21years (true negative).

RESULTS

The intra- and inter-examiner agreements of I3M between the same examiner and two different examiners were 0. 961 (95% CI; 0.907 to 0.983) and 0.929 (95% CI 0.831 to 0.971), indicating good to excellent agreement between them. For the RPV stages, the results of intra-examiner reliability for the same examiner were 0.841 (95% CI 0.767 to 0.899), while the inter-examiner reliability were 0.801 (95% CI 0.744 to 0.968), indicating good agreement. The results of TEM, rTEM, and R values are presented in table 4. The coefficient of reliability (R) values was greater than 95% for I3M measurements for both intra- and inter-examiner agreements.

Table 5 and Figure 3 show the statistical description of the relationship between RPV stages and chronological age in both sexes. Table 6 displays the results of 2x2 contingency tables indicating the discrimination between the subjects aged <21 years and ≥ 21 years by using different I3M cut-off values (0.02 to 0.07). Table 7 displays the results of 2x2 contingency tables for RPV stage 2 for both males and females. Tables 8 and 9 show the quantities derived for different I3M cut-off values in males and females, respectively. Finally, table 10 shows the test results (RPV stage 2) for the discrimination of the subjects.

Table 4. TEM, rTEM and R tests for intra- and inter-examiner agreements

	TEM	rTEM	R
Intra-examiner			
Third molar maturity index	0.07	16.3	0.989
Root Pulp Visibility stages	0.31	25.72	0.950
Inter-examiner			
Third molar maturity index	0.054	12.1	0.972
Root Pulp Visibility stages	0.632	53.63	0.901

TEM, technical error of measurement; rTEM, relative technical error of measurement; R, coefficient of reliability

Table 5. Descriptive statistics of different stages of radiographic visibility of the root pulp in mandibular third molars according to age

Stage	Male					Female				
	Age (in years)					Age (in years)				
	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max
0	22	18.25	0.74	17.33	20.26	20	19.14	1.90	17.41	22.92
1	142	20.72	1.51	18.39	25.81	124	20.85	1.65	18.13	25.66
2	158	25.24	2.20	21.21	29.86	147	24.96	2.09	21.03	29.67
3	39	28.58	0.83	26.31	29.81	52	28.48	1.11	25.68	29.88

SD, Standard deviation; Min, Minimum; Max, Maximum

Figure 3. Boxplot of relationship between chronological age and stages of radiographic visibility of the root pulp in mandibular third molars, according to males and females

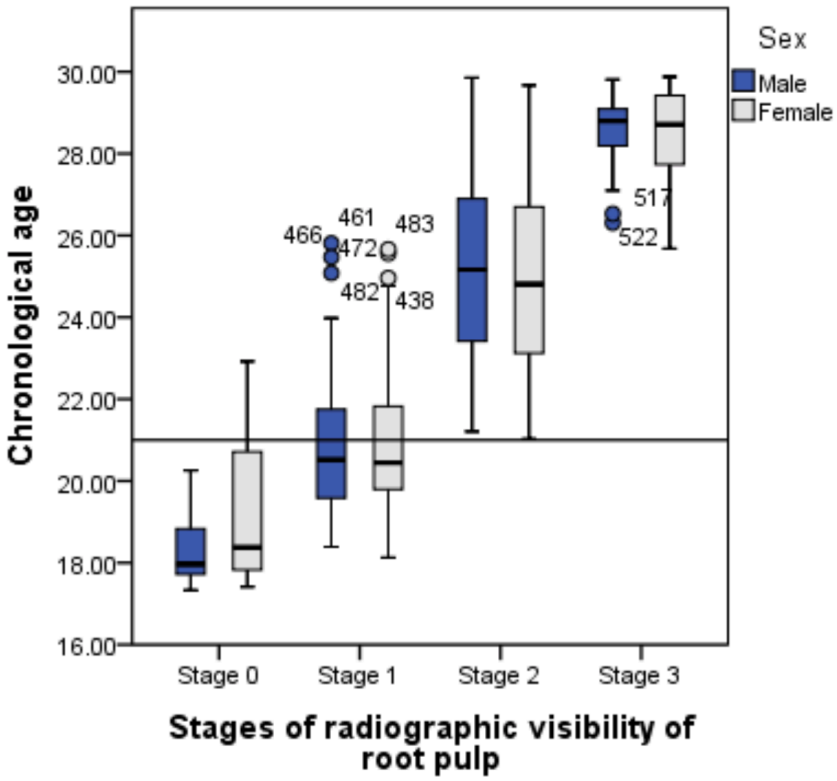


Table 6. Contingency data describing discrimination performance of the test for different cut-off values of third molar maturity index (I3M) in males and females

Males				Females			
Test	Age		Total	Test	Age		Total
	≥ 21 years	<21 years			≥ 21 years	<21 years	
I3M< 0.02	252^{TP}	105^{FP}	357	I3M< 0.02	252^{TP}	89^{FP}	341
I3M ≥ 0.02	0^{FN}	98^{TN}	98	I3M ≥ 0.02	0^{FN}	114^{TN}	114
I3M< 0.03	252^{TP}	106^{FP}	358	I3M< 0.03	252^{TP}	90^{FP}	342
I3M ≥ 0.03	0^{FN}	97^{TN}	97	I3M ≥ 0.03	0^{FN}	113^{TN}	113
I3M< 0.04	252^{TP}	109^{FP}	361	I3M< 0.04	252^{TP}	90^{FP}	342
I3M ≥ 0.04	0^{FN}	94^{TN}	94	I3M ≥ 0.04	0^{FN}	113^{TN}	113
I3M< 0.05	252^{TP}	111^{FP}	363	I3M< 0.05	252^{TP}	94^{FP}	346
I3M ≥ 0.05	0^{FN}	92^{TN}	92	I3M ≥ 0.05	0^{FN}	109^{TN}	109
I3M< 0.06	252^{TP}	112^{FP}	364	I3M< 0.06	252^{TP}	98^{FP}	350
I3M ≥ 0.06	0^{FN}	91^{TN}	91	I3M ≥ 0.06	0^{FN}	105^{TN}	105
I3M< 0.07	252^{TP}	114^{FP}	366	I3M< 0.07	252^{TP}	103^{FP}	355
I3M ≥ 0.07	0^{FN}	89^{TN}	89	I3M ≥ 0.07	0^{FN}	100^{TN}	100

TP, True positive; FP False positive; TN, True negative; FN, False negative

Table 7. Contingency data describing discrimination performance of the test for stage 2 of radiographic visibility of root pulp of mandibular third molars in males and females

Males				Females			
Test	Age		Total	Test	Age		Total
	≥21 years	<21 years			≥21 years	<21 years	
Stages <2	55^{FN}	109^{TN}	164	Stages <2	53^{FN}	91^{TN}	144
Stages ≥2	197^{TP}	0^{FP}	197	Stages ≥2	199^{TP}	0^{FP}	199

TP, True positive; FP False positive; TN, True negative; FN, False negative

Table 8. The quantities derived from 2x2 contingency tables of the test (predicting 21 years) in south Indian males when different cut-off values were used.

Quantities	0.02	0.03	0.04	0.05	0.06	0.07
Accuracy	76.92 (72.7- 80.7)	76.7 (72.5- 80.5)	76.04 (71.8- 79.9)	75.6 (71.3- 79.4)	75.3 (71.1- 79.2)	74.95 (70.7- 78.8)
Sensitivity	100 (98.5- 100)	100 (98.5- 100)	100 (98.5- 100)	100 (98.5- 100)	100 (98.5- 100)	100 (98.5- 100)
Specificity	48.28 (41.2- 55.3)	47.78 (40.7- 54.8)	46.31 (41.2- 55.3)	45.32 (38.3- 52.4)	44.83 (37.8- 51.9)	43.84 (36.9- 50.9)
PPV	70.59 (67.7- 73.2)	70.39 (67.5- 73.1)	69.81 (67.1- 72.4)	69.42 (66.7- 72.1)	69.23 (66.5- 71.8)	68.85 (66.1- 71.4)
NPV	100	100	100	100	100	100
LR+	1.93 (1.69- 2.21)	1.92 (1.68- 2.18)	1.86 (1.64- 2.12)	1.83 (1.61- 2.07)	1.81 (1.60- 2.05)	1.78 (1.58- 2.01)
LR-	0.00	0.00	0.00	0.00	0.00	0.00
PTP	65.9 (62.8- 68.8)	65.8 (62.7- 68.6)	65 (62.1- 67.9)	64.7 (61.7- 67.4)	64.4 (61.5- 67.2)	64 (61.2- 66.8)

Table 9. The quantities derived from 2 x 2 contingency tables of the test (predicting 21 years) in south Indian females when different cut-off values were used.

Quantities	0.02	0.03	0.04	0.05	0.06	0.07
Accuracy	80.44 (76.4- 83.9)	80.22 (76.2- 83.7)	80.22 (76.2- 83.7)	79.34 (75.3- 82.9)	78.46 (74.4- 82.1)	77.36 (73.2- 81.1)
Sensitivity	100 (98.5- 100)	100 (98.5- 100)	100 (98.5- 100)	100 (98.5- 100)	100 (98.5- 100)	100 (98.5- 100)
Specificity	56.16 (49.1- 63.1)	55.67 (48.5- 62.6)	55.67 (48.5- 62.6)	53.69 (46.5- 60.7)	51.72 (44.6- 58.7)	49.26 (42.1- 56.3)
PPV	73.9 (70.7- 76.7)	73.6 (70.5- 76.5)	73.6 (70.5- 76.5)	72.83 (69.8- 75.6)	72 (69.1- 74.7)	70.9 (68.1- 73.7)
NPV	100	100	100	100	100	100
LR+	2.28 (1.95- 2.67)	2.26 (1.93- 2.63)	2.26 (1.93- 2.63)	2.16 (1.86- 2.5)	2.07 (1.8- 2.39)	1.97 (1.72- 2.26)
LR-	0.00	0.00	0.00	0.00	0.00	0.00
PTP	69.5 (66.1- 72.8)	69.3 (65.9- 72.5)	69.3 (65.9- 72.5)	68.4 (65- 71.4)	67.4 (64.3- 70.5)	66.3 (63.2- 69.3)

Table 10. The quantities derived from 2x2 contingency tables of the test (predicting 21 years) in south Indian sample when different stages of radiographic visibility of root pulp of mandibular third molars in males and females

Quantities	RPV Stage 2	
	Males	Females
Accuracy	84.76 (80.6- 88.3)	84.55 (80.2- 88.2)
Sensitivity	78.17 (72.5- 83.1)	78.97 (73.4- 83.8)
Specificity	100 (96.6- 100)	100 (96- 100)
PPV	100	100
NPV	66.46 (61.1- 71.4)	63.19 (57.4- 68.5)
LR+	0.00	0.00
LR-	0.22 (0.17- 0.28)	0.21 (0.17- 0.27)
PTP	100	100

DISCUSSION

According to the age group of medico-legal interest, discriminating against subjects, especially children and young adults, is a common practice in forensic age assessments. Literature indicates evidence of extensive research to predict the attainment of various legal age thresholds, i.e., 12, 14, 15, 16, and 18 years.[7- 11, 23- 25] In addition to these, 21 years has medico-legal importance. According to article 33 of the Turkish Penal code, the legal age of adulthood is 21 years for individuals with hearing impairments.[17, 25] In German criminal courts, if the offender is below 21 years of age, he/she might receive a milder punishment by applying a special juvenile law.[12] According to the Indian marriage law, the legally permissible age of marriage is 21 years for males and 21 years is under consideration for females. [3, 4] Owing to the rise in child marriages, it is essential to determine whether he/she is 21 years old to assess the individual's right to marriage age. It is equally important and relevant to find a methodology to determine whether an individual has attained 21 years of age or not.

In India, forensic dentists receive requests for the assessment of the legal age of 21 years for civil purposes. The authors in this manuscript dealt with civil cases where the youth from the southern states of India, especially Telangana, sought age certificates. The government of Telangana announced multiple schemes, such as the "TS Nirudyoga Bruthi Scheme" and "Bangaru Thalli" scheme. The former provides financial assistance to the unemployed youth, and the latter provides incentives for achieving certain milestones for the female child until she reaches the age of 21.[26, 27]

Much research has been performed on third molar maturation and its application in forensic age assessments. According to the literature, the development of third molars extends into the early twenties. However, to date, no research was available in the literature testing the applicability of I3M for the prediction of 21 years. To the best of our knowledge, this is the first study to test the usefulness of I3M for the legal age threshold of 21 years. We also tried the radiographic visibility of root pulp by Olze et al.,[12], which is applicable after the completed formation of the mandibular third molars.

Third molar maturity index (I3M)

Our results showed that the mineralization of the mandibular third molars finished at 21 years in

males and 22 years in females. We observed that very few female subjects (0.9%) 21 years and above had mandibular third molars with their root apices still open. These findings indicate that the maturation of the mandibular third molars was faster in males than in females, which is in line with the literature. No subjects have shown developing third molars beyond 23 years of age in the studied population.

From a forensic and legal point of view, discrimination performance of the test should show better specificity or fewer subjects who were below 21 years to be wrongly classified as 21 years or older. Considering this, we tested various I3M cut-off values ranging between 0.02 and 0.07. Our study results showed that for I3M< 0.02, the accuracy, sensitivity, specificity, and post-test probability values were 76.92 and 80.44, 100 and 100, 48.28 and 56.16, and 65.9 and 65.9 in both males and females, respectively. However, we did not observe any improvement in the discrimination performance of the test, especially specificity, with the increase in the I3M cut-off value from 0.02 to 0.07. The lower specificity values reflected the presence of a higher percentage of subjects younger than 21 years with fully matured mandibular third molars. Approximately 30% of males and 26% of females younger than 21 had fully developed mandibular third molars. These findings indicate a chance to wrongly identify one in four subjects younger than 21 as above 21.

On the other hand, the likelihood ratios, i.e., the alternative diagnostic measures to discriminate subjects around the legal age threshold, were evaluated.[28] These values are helpful to interpret how many times more or less likely subjects of 21 years or older are to be selected as 21 years and above than individuals below 21 years. Higher LR+ values and lower LR- values mean a good prediction of the probability of the individuals being 21 years and above. Our findings showed poorer LR- values (values close to or equal to 0) for all I3M cut-off values, indicating the probability of correctly identifying individuals aged 21 years or older.

One of the drawbacks of the I3M method for predicting the attainment of 21 years is the inclusion of subjects 23 years and older. It resulted in better sensitivity values as the older subjects whose root apices have closed affect mean measured value of I3M, thereby the performances of the discrimination test.[29]

Radiographic visibility of the root pulp

Our study findings showed almost perfect repeatability and substantial reproducibility. However, these findings are similar to the previous studies.[13- 16] Since the scoring/staging method is a subjective perception of the regressive changes, examiners with experience in forensic age assessments preferred to perform evaluations. Our results further highlighted the need for the training of the examiners and calibration.

According to our study findings, stage 0 could be used as an age marker since all the males were younger than 21 years of age; for females, the results were not as discriminatory as for males since 20% of females marked stage 3 were older than 21. Stage 2 did not account for better discriminatory capacity; 61.3% of males and 60% of females were younger than 21. On the other hand, stages 2 and 3 proved valuable markers since all the males and females in these respective stages (2 and 3) were older than 21. These findings were similar to Olze et al. [12], which stated that stages 2 and 3 safely indicate age over 21 years. In addition, our results were partly identical to that of Pérez-Mongiovi et al. [15], in which they reported that stage 2 was not a helpful marker to indicate age over 21 years since it resulted in approximately 15% errors. According to them, stage 3 has better discriminatory capacity in both sexes. In another study by Guo et al. [18] in the Northern Chinese sample, subjects found to be in stages 2 and 3 were at least 21 years of age and older.

There are many ways to report a medical age assessment in medico-legal practice. They include presenting the most probable age, the minimum age, or the probability of the person being below or above the age of medico-legal importance.[30] The concept of minimum age is easy to understand, conservative, and should be applied when one needs the highest standard of proof.[31] It gives the benefit of the doubt to younger people. The minimum age concept shows that stages 2 and 3 have provided strong evidence to indicate the age over 21. Stage 2 indicated a minimum age of 21.21 years and 21.03 years, while stage 3 indicated 26.31 years and 25.68 years in both males and females, respectively. The sensitivity and specificity values for RPV stage 2 were better. The accuracy, sensitivity, and specificity for stage 2 were 84.76 and 84.55, 78.17 and 78.97, and 100 and 100 in both males and females, respectively.

The post-test probability was 100% for both sexes, i.e., the grading of stage 2 indicated the possibility for the subject to be over 21 was 100%.

One drawback of the radiographic visibility of the root pulp is its limited application to the fully developed third molars. It is rare to see fully formed mandibular third molars with closed roots under the age of 18 years.[12] We did not apply this method in a 100% sample of both sexes of 16 years age group, 80% males and 77.5% females in 17 years, 37.5% males and 45% females in 18 years; and 20% males and 50% females in 19 years age group. Timme et al. [19], in their study, have applied this method in only 36-46% of radiographs of the total sample. In another study by Guo et al.,[18], approximately 20% of the radiographs could not be evaluated due to fused roots, narrowed furcation, missing third molars, and insufficient quality. In the present study, we excluded the radiographs falling under the above-mentioned criteria, which resulted in a non-balanced distribution of the sample, especially in the younger age groups. It is a significant concern for this method. Therefore, we recommend using a larger sample size to predict legal age thresholds of 18 and 21 years, especially in the younger age groups.

CONCLUSIONS

To the best of our knowledge, this is the first study to investigate the use of the I3M method to predict the legal age threshold of 21 years in emerging adults of south Indian origin. Based on our results, the following observations were made;

1. I3M cut-off values ranging from 0.02 to 0.07 resulted in a more significant percentage of false positives. Subjects who were 21 years and older were correctly identified. However, there is a chance of wrongly identifying one in four subjects younger than 21 as above 21.
2. When the minimum age concept was applied, RPV stages 2 and 3 provided substantial evidence to indicate an age above 21. In addition, stage 2 resulted in better sensitivity values than stage 3.

To conclude, the I3M method cannot state the attainment of the legal age of 21 years, owing to a greater percentage of false positives. However, RPV stage 2 can say that a subject is over 21 years old in both sexes.

REFERENCES

1. Norelli, G.A., Pinchi, V. Odontologia Forense—Medicina Legale in Odontoiatria—Guida Alla Valutazione del Danno Odontostomatologico; Piccin Editore: Milan, Italy, 2010.
2. Aggrawal, A. Age estimation in the living- Some Medicolegal considerations. Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology [Serial online], 2000;1(2):about 23 pages. Available from: https://anilaggrawal.com/ij/vol_001_no_002/ug001.html. (Last accessed; January 10, 2022).
3. Ranjana Kumari. Faceoff Part I: Raising marriage age of girls. Times of India. Available from: https://timesofindia.indiatimes.com/india/faceoff-part-i-raising-marriage-age-of-girls-why-should-a-girl-marry-at-18-while-a-boy-has-till-21-to-make-a-better-life/articleshow/88455429.cms (Last accessed; January 10, 2022).
4. Express News Service. Bill to raise age of marriage for girls to 21 introduced in Lok Sabha. Available from: https://indianexpress.com/article/india/bill-to-raise-age-of-marriage-for-girls-introduced-in-lok-sabha-7683455/ (Last accessed; January 10, 2022).
5. Manjunatha, B.S., Soni, N.K. Estimation of age from development and eruption of teeth. J Forensic Dent Sci. 2014;6(2):73-6.
6. Moorres, C.F.A., Fanning, E.A., Hunt, E.E. Age variation of formation stages for ten permanent teeth. *J Dent Res*. 1963;42(6):142–149.
7. Cameriere, R., Ferrante, L., De Angelis, D., Scarpino, F., Galli, F. The comparison between measurement of open apices of third molars and Demirjian stages to test chronological age of over 18 year olds in living subjects. *Int J Legal Med*. 2008;122(6):493-7.
8. Jiménez, L.G., Palacio, L.A.V., De Luca, S., Vasquez, Y.R., Capellán, M.C., Cameriere, R. Validation of the third molar maturity index (I_{3M}): study of a Dominican Republic sample. J Forensic Odontostomatol. 2019;37(3):27–33.
9. Pinchi, V., Norelli, G.A., Pradella, F., Vitale, G., Rugo, D., Nieri, M. Comparison of the applicability of four odontological methods for age estimation of the 14 years legal threshold in a sample of Italian adolescents. J Forensic Odontostomatol. 2012;30(2):17-25.
10. Balla, S.B., Banda, T.R., Galic, I., N, N.M., Naishadham, P.P. Validation of Cameriere's third molar maturity index alone and in combination with apical maturity of permanent mandibular second molar for indicating legal age of 14 years in a sample of South Indian children. *Forensic Sci Int*. 2019;297:243-248.
11. Balla, S.B., Chinni, S.S., Galic. I., Alwala, A.M., Machani, P., Cameriere, R. A cut-off value of third molar maturity index for indicating a minimum age of criminal responsibility: Older or younger than 16 years? *J Forensic Leg Med*. 2019;65:108-112.
12. Olze, A., Solheim, T., Schulz, R., Kupfer, M., Schmeling, A. Evaluation of the radiographic visibility of the root

pulp in the lower third molars for the purpose of forensic age estimation in living individuals. *Int J Legal Med*. 2010;124(3):183-6.
13. Lucas, V.S., McDonald, F., Andiappan, M., Roberts, G. Dental Age Estimation-Root Pulp Visibility (RPV) patterns: A reliable Mandibular Maturity Marker at the 18 year threshold. *Forensic Sci Int*. 2017;270:98-102.
14. Al Qattan, F., Alzoubi, E.E., Lucas, V., Roberts, G., McDonald, F., Camilleri, S. Root Pulp Visibility as a mandibular maturity marker at the 18-year threshold in the Maltese population. *Int J Legal Med*. 2020;134(1):363-8.
15. Pérez-Mongiovi, D., Teixeira, A., Caldas, I.M. The radiographic visibility of the root pulp of the third lower molar as an age marker. *Forensic Sci Med Pathol*. 2015;11(3):339-44.
16. Gok, E., Fedakar, R., Kafa, I.M. Usability of dental pulp visibility and tooth coronal index in digital panoramic radiography in age estimation in the forensic medicine. *Int J Legal Med*. 2020;134(1):381-92.
17. Akkaya, N., Yılancı, H.Ö., Boyacıoğlu, H., Göksülük, D., Özkan, G. Accuracy of the use of radiographic visibility of root pulp in the mandibular third molar as a maturity marker at age thresholds of 18 and 21. *Int J Legal Med*. 2019;133(5):1507-15.
18. Guo, Y.C., Chu, G., Olze, A., Schmidt, S., Schulz, R., Ottow, C., Pfeiffer, H., Chen, T., Schmeling, A. Application of age assessment based on the radiographic visibility of the root pulp of lower third molars in a northern Chinese population. *Int J Legal Med*. 2018;132(3):825-9.
19. Timme, M., Timme, W.H., Olze, A., Ottow, C., Ribbecke, S., Pfeiffer, H., Dettmeyer, R., Schmeling, A. The chronology of the radiographic visibility of the periodontal ligament and the root pulp in the lower third molars. *Sci Justice*. 2017;57(4):257-61.
20. Pyata, J.R., Kandukuri, B.A., Gangavarapu, U., Anjum, B., Chinnala, B., Bojji, M., Gurram, A., Balla, S.B. Accuracy of four dental age estimation methods in determining the legal age threshold of 18 years among South Indian adolescents and young. *J Forensic Odontostomatol*. 2021;3(39):2-15.
21. Balla, S.B., Ankisetti, S.A., Bushra, A., Bolloju, V.B., Mir Mujahed, A., Kanaparthi, A., Buddhavarapu, S.S. Preliminary analysis testing the accuracy of radiographic visibility of root pulp in the mandibular first molars as a maturity marker at age threshold of 18 years. *Int J Legal Med*. 2020;134(2):769-74.
22. M, S., Balla, S.B., Chinni, S.S., K, S.P.R., Gopalaiah, H., C, P., R, N.R. Examination of the radiographic visibility of the root pulp of the mandibular second molars as an age marker. *Int J Legal Med*. 2020;134(5):1869-73.
23. Angelakopoulos, N., Galić, I., Balla, S.B., Kiş, H.C., Gómez Jiménez, L., Zolotenkova, G., Mohd Yusof, M.Y.P., Hadzić Selmanagić, A., Pandey, H., Palmela Pereira, C., Nóbrega, J.B.M., Hettiarachchi, K., Mieke, S.M., Kumagai, A., Gulsahi, A., Zelić, K., Marinković, N., Kelmendi, J., Bianchi, I., Soriano Vázquez, I., Spinas, E., Velezmoro-Montes, Y.W., Oliveira-Santos, I., De Luca, S., Arrais Ribeiro, I.L., Moukarzel, M., Cameriere, R. Comparison of the third molar maturity index (I_{3M}) between left and right lower third molars to assess the age of majority: a multi-ethnic study sample. *Int J Legal Med*. 2021;135(6):2423-36.
24. Wang, M., Wang, J., Pan, Y., Fan, L., Shen, Z., Ji, F., Tao, J. Applicability of newly derived second and third molar maturity indices for indicating the legal age of 16 years in the Southern Chinese population. *Leg Med (Tokyo)*. 2020;46:101725.
25. Yılancı, H.O., Akkaya, N., Bulut, G., Göksülük, D., Bulut, H., Cameriere, R. Assessment of second (I_{2M}) and third (I_{3M}) molar maturity indices individually and in combination (I_{2M+3M}) and the sum of the seven mandibular teeth indices (S) for the legal age thresholds of 12 and 15 years in a Turkish sample. *Aust J For Sci*. 2021. doi: 10.1080/00450618.2020.1865454.
26. Telangana Unemployment Allowance scheme 2022: TS Nirudyoga Bruthi Scheme. Available from; https://icdsupweb.org/telangana-unemployment-allowance-scheme/ (Last accessed; September 29, 2022).
27. Telangana: Bangaru Talli scheme. Telangana, Women and Child Welfare. Available from: https://www.theindianiris.com/telangana-bangaru-talli-scheme/ (Last accessed; September 29, 2022).
28. Deeks, J.J., Altman, D.G. Diagnostic tests 4: likelihood ratios. *BMJ*. 2004;329(7458):168-9.
29. Liversidge, H.M., Marsden, P.H. Estimating age and the likelihood of having attained 18 years of age using mandibular third molars. *Br Dent J*. 2010;209(8):E13.
30. Mostad, P., Schmeling, A., Tamsen, F. Mathematically optimal decisions in forensic age assessment. Int J Legal Med. 2021. doi: 10.1007/s00414-021-02749-y.
31. Schmeling, A., Dettmeyer, R., Rudolf, E., Vieth, V., Geserick, G. Forensic Age Estimation. *Dtsch Arztebl Int*. 2016;113(4):44-50.