Dentinal translucency and width of cementum: predicting the age over 55 years in South Indian adults using extracted sectioned teeth

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The authors declare that they have no conflict of interest.

KEYWORDS

Dental age estimation,
Root dentin translucency,
Width of cementum
Age threshold,
Probability

J Forensic Odontostomatol
2022. Aug;(40): 2-10:20
ISSN :2219-6749

ABSTRACT

The main intention of this article was to evaluate the reliability of root dentine translucency (RDT) and the width of cementum (CW) in indicating the age over 55 years in forensic and criminal investigations. 600 non-restored, single rooted teeth (300 males and 300 females) which were extracted for periodontal or orthodontic reasons were collected. Each tooth was sectioned longitudinally until the desired thickness (250 µm) was obtained. Both the length of the RDT and CW were measured from these unstained ground sections of teeth using ImageJ computer software. Pearson's correlation coefficient indicated a very strong and positive correlation for RDW and CW with age in both sexes. Cut-off values of RDT= 7.07 and CW= 52.06 were obtained using the maximum Youden's index. The value for the area under the curve (AUC) was 0.987 for RDT and 0.910 for CW was seen as indicating a very high discrimination. The performance of these cut-off values was tested in a separate sample of sectioned teeth (n= 300) and was analyzed using contingency tables in both sexes. The sensitivity was 88.2% and 92.3%, while specificity was 98.9% for RDT in males and females. For CW, the sensitivity was 96.1% and 90.3%, and specificity was 76.7% and 74.4% in males and females, respectively. Bayes post-test probability was 98.9% for RDT in both sexes, while 80.5% in males and 78% in females, for CW. Based on our study findings, it can be concluded that both variables have performed well in predicting the age over 55 years. Further research concerning the radiographic study of secondary dentine deposition to predict legal age thresholds would be a great benefit for living adults who require age estimation in civil proceedings.

INTRODUCTION

Establishing identity to unknown bodies or skeletal remains is of paramount importance in the legal context of forensic and anthropological practice.[1] To this end, estimating the age of the individual can be considered as the prime factor and it represents a key feature leading to the identification of the unknown. Depending on the context of the investigation, the forensic experts use either osseous or dental based methods. Even though skeletal methods are indicated to be reliable for age estimation, their poor resistance to the taphonomic process and variability in their maturation under the influence of environmental factors made experts look for alternative parameters.[2] Some dental tissues are extremely resilient,

resisting mechanical, chemical, and thermal stimulation even in the most adverse situations.[3, 4] Following the completion of the developmental changes occurring in teeth, one must rely on the changes in dental hard tissues for estimation of age. A variety of age estimation approaches have been proposed by researchers in the past. In 1925, Bodecker initially observed a relationship between secondary dentine apposition and increasing age.[5] Later, in 1950, Gustafson studied the regressive alterations in teeth and it was the first scientific method for estimation of age in adults.[6] The numerous approaches used to estimate age in adults have arisen as a result. Among them, root dentine translucency (RDT) was found to be reliable by many authors.[7-10] Similarly, studies also found a positive correlation between the cementum thickness and age, and its role in age assessment.[11-13]

In medico-legal cases, age is a valuable criterion to determine essential services, systems and entitlements. The major issues in age estimation in the living concern illegal migration of refugees, adoption, imputability (14, 16, 18 and 21 years in different countries), and old age pension (50, 55, 60, 65 years in different countries).[14] More often forensic experts are called for evaluation of the true age of adults for old age pension, access to medical care or other civil purposes (retirement). Unlike children, there are no age thresholds in adults that had medico-legal importance. However, in some circumstances, the services of doctors/forensic experts were utilized for evaluation of the true age of adults for multiple purposes, for example if they have attained the age of 55 years.[14, 15]Similarly, in criminal investigations the police often consult forensic experts to determine the age of unidentified human remains. In such instances, it is the duty of the experts to provide an age range that helps the police narrow down the search. Therefore, it is important to investigate the regressive changes in teeth with specific emphasis on the different age thresholds in adults.

To date, and to the best of our knowledge, no study has evaluated the specificity and sensitivity indices, and the probability in evaluating the age group of 55 years. The present study aims at deriving cut-off values separately by means of RDT length and CW, and to verify the performance of newly determined cut-off values in age indication over 55 years in a new sample.

MATERIAL AND METHODS

Sample

600 freshly extracted single rooted permanent teeth from healthy volunteers (300 males & 300 females) aged between 15 and 75 years were collected from the department of Oral and Maxillofacial Surgery and from private clinical practitioners. The extracted teeth were divided into six equal age groups, with each age group ranging up to 10 years span and consisting of 50 teeth with an equal number of male and female samples. The age, sex, reason for extraction and from whom teeth were extracted were noted. Teeth extracted for orthodontic or prosthodontic reasons and those who complained of mobile teeth due to periodontitis were collected. Teeth with pathologies like caries, hypercementosis, trauma and teeth with loss of tooth substance due to tooth-to-tooth contact due to pathological causes were excluded. After obtaining institutional ethical committee clearance (SVSIDS/OP/4/2018) and informed consent from the patients, extracted teeth were collected.

Study sample distribution

The Study Group sample (n=600) was randomly split into two sub-samples. One of the sub-samples was referred to as the test sample, while the other was referred to as the validation sample. A cut-off value was calculated, which is known to result in the best overall performance of the discrimination test. Subsequently, the second sample was utilized to verify the feasibility of the cut-off values, and to see if the person had attained the age of 55. For the whole sample, age and sex distribution is shown in Table 1.

Preparation of tooth specimen

The teeth were preserved in 10% neutral buffered formalin immediately after extraction to prevent dehydration. Later, each tooth was sectioned longitudinally (in buccolingual direction) using a diamond disc followed by Arkansas stone until desired thickness (250 µm) was obtained. The slides were washed in xylene and then put in DPX to view under a light microscope. Cover slips were placed for viewing using a stereomicroscope.

Table 1. Age and gender distribution of the overall (test & validation) sample

Age groups	Test sample			Validation sample		
	Males	Females	Total	Males	Females	Total
15- 25	25	25	50	25	25	40
26- 35	25	25	50	25	25	40
36- 45	25	25	50	25	25	40
46- 55	25	25	50	25	25	40
56- 65	25	25	50	25	25	40
66- 75	25	25	50	25	25	40
Total	150	150	300	150	150	300

Measurements

Cementum from the mid-root section was observed under light microscope and digital images were captured using Olympus Preogress C3 camera. Teeth with indistinct, invisible cementum were eliminated. The width of the cementum (CW) was measured between DCJ (dentino-cementum junction) to the outer surface of the cementum (**Figure** 1). The width of the cementum (X) was measured between DCJ (dentino-cemental junction) to the outer surface of cementum. Subsequently the width between two adjacent incremental lines (Y) was measured and the number of incremental lines (N) in each section was calculated using the formula: N= X/Y. Finally, the estimated age of individual (E) was calculated by adding average eruption age (T) in years of each tooth with total number of incremental lines. E= N+T. To measure the length of the root dentinal translucency (RDT), sections were observed and photographed under stereomicroscope (**Figure** 2). Using the image analysis software the apical translucent dentine (T) length was measured. In every tooth section measured, the length of dentinal translucency was measured from the coronal most extent to apical most extent of the dentinal translucency. Modified Bang and Ramm's formula was applied to all measurements and the age of the individual was estimated.

T= apical translucent dentine length

Linear regression:

$T \geq 9$ mm

Age= 35.5619+ (3.4828 x T).

Quadratic regression:

If $T \leq 9$ mm

Age = 29.9074+ (7.4507 x T) + (-0.4369 x T^2). All linear measurements were carried out with the help of computerized image processing programme (ImageJ, U.S. National Institute of Health, Bethesda, Maryland).

All extracted tooth samples were assessed by an experienced examiner who was unaware of the subjects' details. To check for any intra-examiner and inter-examiner variations, 50 extracted teeth were randomly selected and were re-assessed after an interval of 2 weeks.

Data management and Statistical analysis

The age, sex, RDT, and CW values of each tooth specimen were noted in a Microsoft Excel 2016 spreadsheet (Microsoft Office 2003, Microsoft, Redmond, WA). To avoid any future misunderstanding, each tooth specimen was assigned an identifying number, and all of the specimens' information was recorded against that number. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 20.0 version for Windows (SPSS Inc., Chicago, IL, USA) and the significance level was set at 5% (P0.05). The intra- and inter-examiner variations were determined using the intra-class correlation coefficient (ICC). A binary logistic, generalized linear regression model was used on the test sample to validate the relevance of the factors (RDT, CW, and sex) on the discriminating of participants over 55 years old from those under 55 years old. The efficacy of the RDT and CW values was tested using a receiver operating characteristic (ROC) curve (a graphical figure that shows the entire trade-off between sensitivity and specificity over a succession of cut-off points). The discrimination test's greatest performance was determined using a single statistic, Youden's index (J), which is a function of sensitivity and specificity. RDT and CW cut-

off values were determined using the largest Youden's index.[16]The performance of the generated RDT and CW cut-off values was assessed using a 2X2 contingency table, which shows the number of true positives, false positives, true negatives and false negatives. We calculated the percentage of correct categorization (Ac), sensitivity (Se), specificity (Sp), predictive values, and likelihood ratios. Pre- and post-test probability of being 55 years or older were computed to distinguish between people aged 55 years and those aged >55 years. The likelihood that there are people in a given group who are 55 years or older before a diagnostic test is performed is known as pre-test probability (p_o). Post-test probability (PTP), on the other hand, can be defined as the likelihood that a person is 55 years or older when the diagnostic test identifies the condition. The standard form of Bayes' theorem for a positive result should be written as:

Post - test probability= $p_o \times Se / (p_o \times Se) + (1- p_o) \times (1- Sp)$

p_o is the pre-test probability of being 55 or older in this equation. It was evaluated using data from the Census of India website: https://www.censusindia.gov.in/2011census/C-series/C-13.html. It was computed using demographic data from the 2011 census to determine the proportion of individuals aged 55 to 75 years old and those aged 15 to 75 years old. According to this, the proportions for males and females were determined as 50% for males and 50% for females, respectively.

Figure 1. Images of sound upper right lateral incisor showing the incremental cemental lines under microscopic magnification

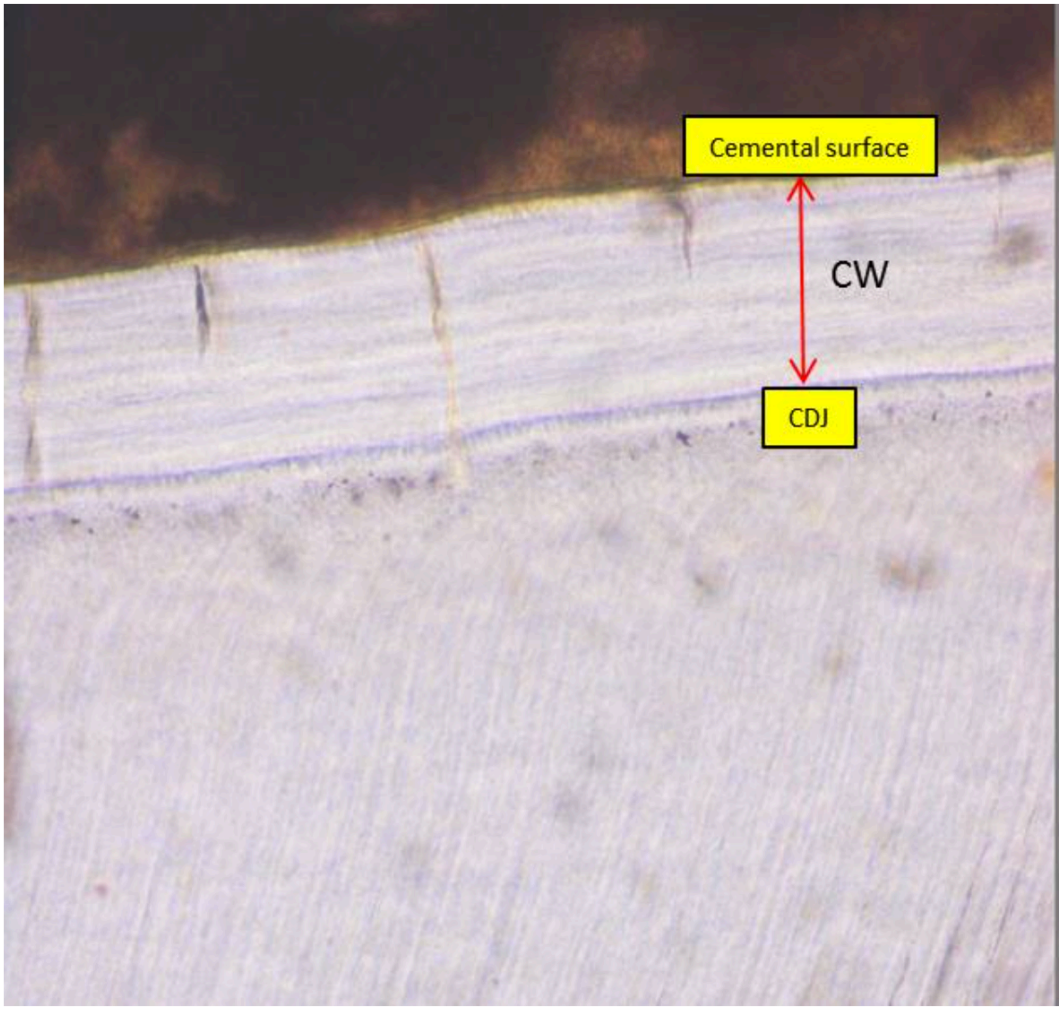
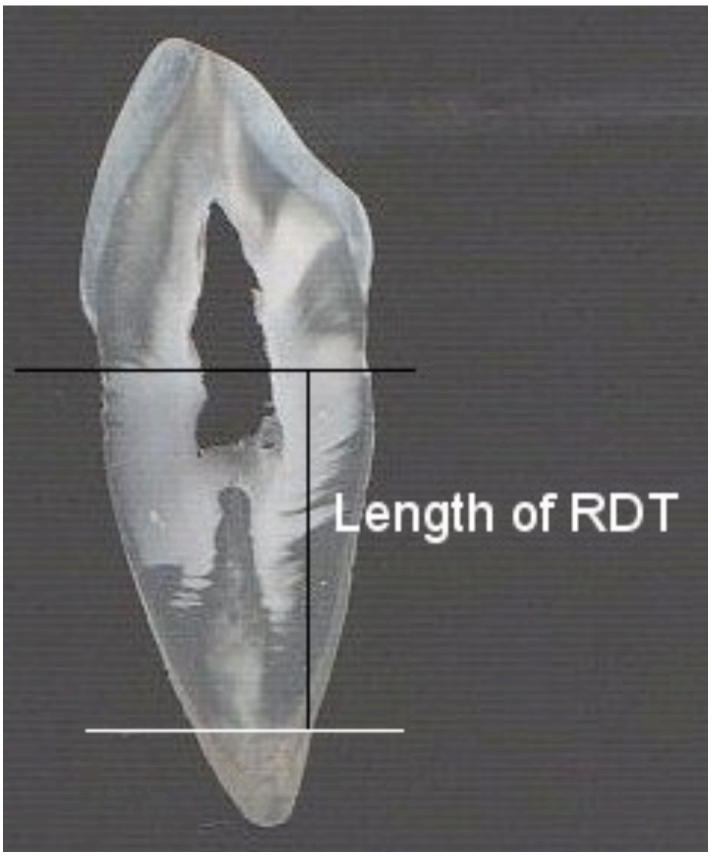


Figure 2. Measurement of the length of the root dentin translucency using ImageJ computer software



RESULTS

The total sample's average age was 44.78 + 17.4 years. Males and females, on the other hand, were 44.67 + 17.4 years and 44.89 + 17.5 years old, respectively. According to Pearson's correlation statistics, there was a significant and extremely strong relationship between age and RDT ($R2$= 0.949), CW ($R2$= 0.930). (Table 2 and Figures 3 & 4). The intra- and inter-examiner agreement ICC values for RDT were 0.917 and 0.891, respectively. For CW, the ICC for intra- and inter-examiner agreement was 0.905 and 0.872, respectively.

Except for sex (p=0.068), all of the predictor factors were statistically significant in the logistic regression model (Table 3). The overall model explained 0.644 (Cox & Snell R Square) to 0.890 (Nagelkerke R Square) of the variations in the ≥55 years and <55 categories.

Using dentine translucency, the area under the curve (AUC) was 0.987 (Figure 5). For the maximum value of the Youden index, which was 0.893 for the cut-off value of DT= 7.07, a cut-off value of dentine translucency was developed to differentiate that persons are ≥55 years or <55 years.

Using width of cementum, the area under the curve (AUC) was 0.910 (Figure 6). For the maximum value of the Youden index, which was 0.688 for the cut-off value of CW= 52.06, a cut-off value of cementum width was developed to differentiate whether persons are ≥55 years or <55 years.

Tables 4 and 5 provide the results of the contingency tables, which illustrate how the cut-off values performed in a different sample (n=300). For males, the Ac, Se, Sp, LR+, LR-, PPV, and NPV were 95.3%, 88.2%, 98.9%, 87.35%, 0.12%, 97.8%, and 94.2%, respectively, using the root dentine translucency (RDT>7.07). Females scored 96.6%, 92.3%, 98.9%, 90.46, 0.08, 97.9%, and 96%, respectively. In both males and females, the calculated Bayes post-test probability was 98.9% (Table 6).

The Ac, Se, Sp, LR+, LR-, PPV, and NPV for males using the other predictor, CW > 52.06, were 83.3%, 96.1%, 76.7%, 4.14, 0.05, 68.1%, and 97.4%, respectively. Females scored 80%, 90.3%, 74.4%, 3.54, 0.13, 65.2%, and 93.5%, respectively. Males had an estimated Bayes post-test probability of 80.5%, whereas females had an estimated Bayes post-test probability of 78% (Table 6).

Table 2. Pearson's correlation between the age and the parameters tested (RDT & CW) in both males and females

Parameters	Sex (n)	Correlation	p- value
RDT	Males (150)	0.978	0.000*
	Females (150)	0.920	0.000*
CW	Males (150)	0.939	0.000*
	Females (150)	0.921	0.000*

*Statistically significant (p<0.05)

Figure 3. Scatter plot showing the relation between the age and the length of the root dentin translucency in males and females

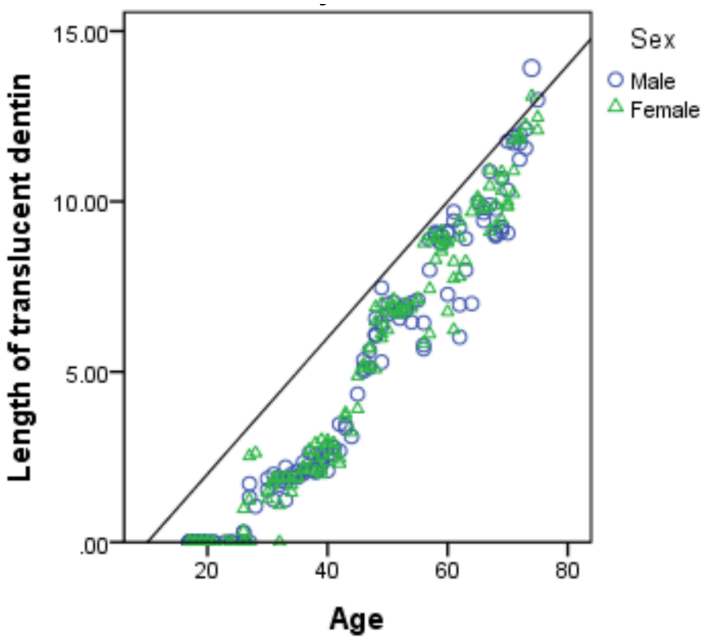


Figure 4. Scatter plot showing the relation between the age and width of the cementum in males and females

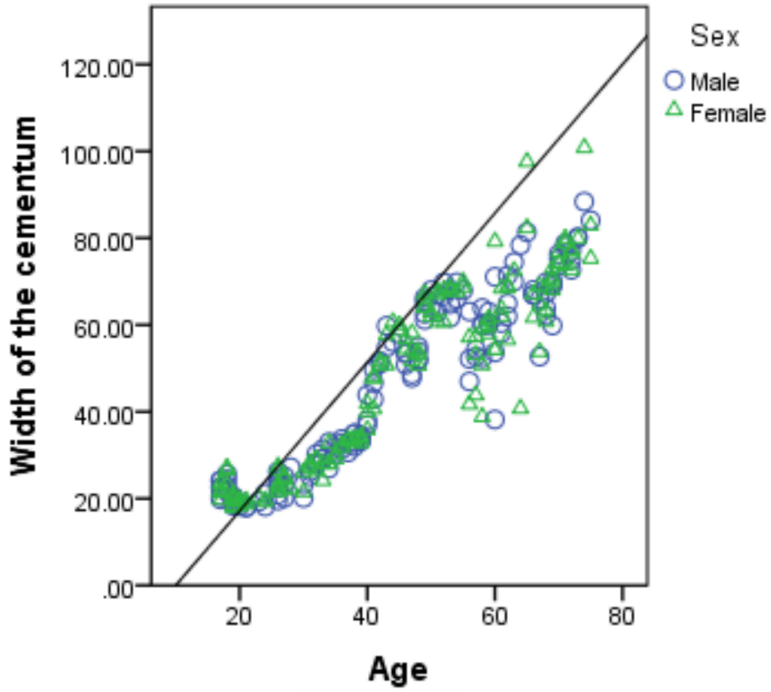


Table 3. Parameter estimates of the RDT and CW and sex as explanatory variables and ≥55 years and <55 years age as dichotomous dependent variable on Logistic Regression

	B	Std. Error	Wald	df	Sig.
Sex	-0.583	0.378	3.129	1	0.068
RDT	1.986	.367	29.232	1	0.000*
CW	-2.031	.440	31.578	1	0.000*
Constant	-12.062	3.102	15.125	1	0.000*

*Statistically significant (p<0.05)

Figure 5. The receiver operating characteristic curve for indicating the legal age of 55 years using length of the root dentin translucency

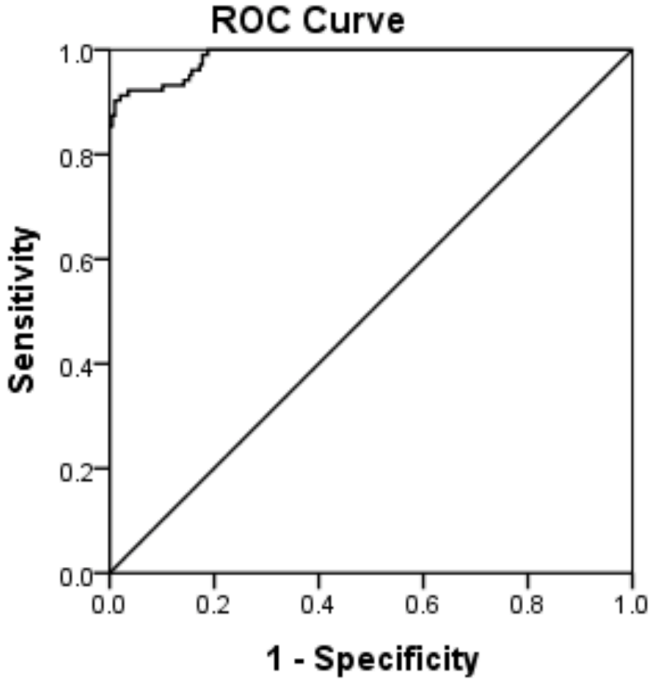


Figure 6. The receiver operating characteristic curve for indicating the legal age of 55 years using width of the cementum

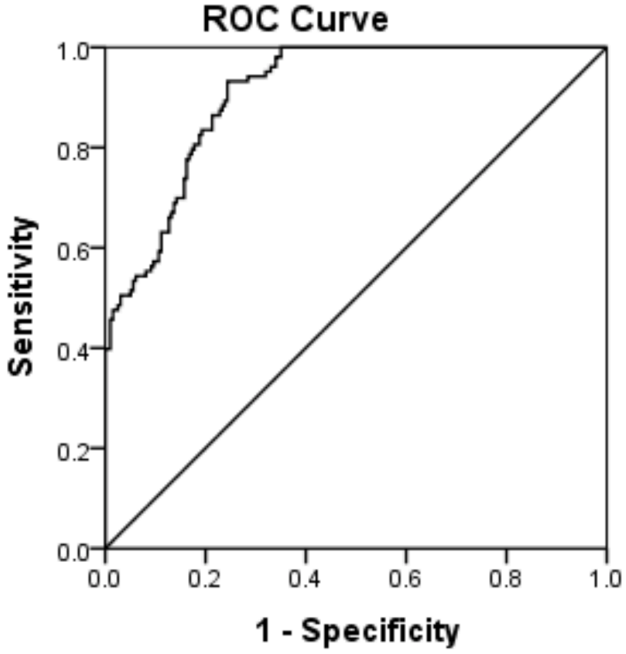


Table 4. Contingency table describing discrimination performance of the test (≥ 55 years and < 55 years) for RDT ≥ 7.07 in males and females

Test		Age Category		Total
		<55 years	≥55 years	
1.		**Males**		
DT≥7.07	**≥7.07**	1^{FP}	45^{TP}	46
	<7.07	98^{TN}	6^{FN}	104
Total		99	51	150
2.		**Females**		
DT≥7.07	**≥7.07**	1^{FP}	48^{TP}	49
	<7.07	97^{TN}	4^{FN}	101
Total		98	52	150

TP, true positive; FN, false negative, FP, false positive, TN, true negative.

Table 5. Contingency table describing discrimination performance of the test (≥ 55 years and < 55 years) for CW ≥ 52.06 in males and females

Test		Age Category		Total
		<55 years	≥55 years	
1.		**Males**		
CW≥52.06	**≥52.06**	23^{FP}	49^{TP}	72
	<52.06	76^{TN}	2^{FN}	78
Total		99	51	150
2.		**Females**		
CW≥52.06	**≥52.06**	25^{FP}	47^{TP}	72
	<52.06	73^{TN}	5^{FN}	78
Total		98	52	150

TP, true positive; FN, false negative, FP, false positive, TN, true negative.

Table 6. Parameter estimates of the RDT and CW and sex as explanatory variables and ≥55 years and <55 years age as dichotomous dependent variable on Logistic Regression

	Root Dentin Translucency		Width of the Cementum	
	Males	**Females**	**Males**	**Females**
Sensitivity	88.2 (76.1- 95.5)	92.3 (81.4- 97.8)	96.1 (86.5- 99.5)	90.3 (78.9- 96.8)
Specificity	98.9 (94.5- 99.9)	98.9 (94.4- 99.9)	76.7 (67.2- 84.6)	74.4 (64.6- 82.7)
Accuracy	95.3 (90.6- 98.1)	96.6 (92.3- 98.9)	83.3 (76.3- 88.9)	80 (72.7- 86.1)
LR+	87.35 (12.4- 615.6)	90.46 (12.85- 636.81)	4.14 (2.88- 5.94)	3.54 (2.5- 5.03)
LR-	0.12 (0.06- 0.25)	0.08 (0.03- 0.20)	0.05 (0.01- 0.20)	0.13 (0.06- 0.30)
PPV	97.8 (86.4- 99.6)	97.9 (87.2- 99.7)	68.1 (59.7- 75.3)	65.2 (56.9- 72.7)
NPV	94.2 (88.5- 97.1)	96 (90.4- 98.4)	97.4 (90.6- 99.3)	93.5 (86.2- 97.1)
Bayes PTP	98.9 (92.5- 99.8)	98.9 (92.8- 99.8)	80.5 (74.2- 85.6)	78 (71.4- 83.4)

LR+, positive likelihood ratio; LR-, negative likelihood ratio; PPV, Positive predictive value; NPV, Negative predictive value; Bayes PTP, Bayes post-test probability.

DISCUSSION

Root Dentine translucency

In the past, age estimation studies in adults focused on one of the regressive changes i.e. quantification of dentine translucency on extracted teeth.[10, 17] However, variations in the evaluation of this age-related change were observed in the literature with respect to their analysis (sectioned or unsectioned tooth),[18, 19] grading or the measurement of the variable,[20] measuring the length or area of the translucency,[21, 22] and the examination of the variable i.e. caliper based or the digital approach.[8, 23, 24] When dentine translucency was studied on the unsectioned extracted teeth, high variation was seen, especially the correlation between age and translucency (0.34 to 0.97).[18, 25]

Jigna S Shah et al. examined sectioned teeth for the clear appearance of dentine translucency on the tooth sections.[18, 26] Literature indicated a high correlation between the age and translucency, particularly in sectioned teeth.[8, 26, 27]On this point, our results also have shown very high correlation. On comparing results of this study with the other Indian studies alone,[27-29] the correlation values both in males and females were greater. Even though, Acharya and Vimi[27] showed a moderate correlation of translucency length with age, their findings were not truly reflected in our study. The possible difference between the results of our study to previous ones[27-29] is that; firstly, 300 sections used in the present study (relatively larger sample size) and secondly, coverage of wider range from younger age groups to the older with reasonably equal distribution of age groups and number of sections as per sex in each age group.

Width of the cementum

In addition to dentine, cementum is another calcified dental tissue. Unlike enamel, it is synthesized continuously and maintained throughout all stages of adult life.[30] Few have considered the analysis of cementum apposition and thickness as a better parameter for age estimation due to its distinctive location in the alveolar process.[31] Literature evidence has also suggested that the migration of cementum coronally in impacted teeth can also be helpful in age estimation.[32, 33] Studies in the literature have indicated that both the cemental thickness and cemental annulations are best correlated with age. When Kasetty et al.[13] studied both these variables in estimating age, they have reported a significant correlation. However, cemental thickness was statistically more significant than cemental annulations. Similar findings were also reported by Stein et al.[12] that quantitation of cemental annulations as a moderately reliable means of age estimation in adults. Additionally, Dias et al.[34] highlighted the difficulties that one can face while studying cemental annulations such as variability in their thickness, blurry or indistinct annulations, overlay of the single annulation at varying levels (could lead to misinterpretation of two lines) etc. These may result in bias while making the count and may account for moderate correlation of cemental annulations with age. Considering all these studies into account, the authors in this study examined the cemental thickness.

The present study also aimed to identify the relationship between the CW and age. Variables revealed a strong, positive and statistically significant correlation, which correlates with the findings of Priya Gupta et al.[11], Jigna et al.[10] and Kasetty et al.[13] Variations in the correlations between the age and cementum thickness have been reported in the literature based on the different areas (the apex or one third of the root length) and type of tooth (maxillary or mandibular) analyzed. Solheim[35] indicated that the cemental thickness analyzed at the one third of root length as most accurate predictor of the age. Similarly, when we analyzed the cemental thickness at middle third of the root, we observed a very high correlation, both in males and females. However, these findings were contrary to that of Jigna et al.[26] and Kasetty et al.[13] where they found apical thickness of cementum as a better predictor of age in adults.

Misclassifications and probability

When it comes to the prediction of attainment of age thresholds in medico-legal cases or in criminal proceedings, it is of supreme importance that age misclassifications should be avoided or kept to a minimum. In children and sub-adults, who were involved in criminal proceedings, only age estimation methods that have exhibited high specificity and very probability (>90%) must be used.36 However, under civil law, the context of specificity and probability will be different; a

probability of around 51 percent may be adequate for determining age in civil procedures.37 In the current study, when RDT was used to indicate age over 55 years, a probability of 98.9% was observed for both males and females, respectively. On the other hand, CW has produced lesser probability values which is 80.5% in males and 78% in females. However, in the context of civil proceedings, both these variables have performed well and therefore can be applied for estimation of age in adults, especially in evaluating the age threshold of 55 years in the population.

Limitations and recommendations

Each method of age estimation in practice has its own set of limitations and benefits. One of the main limitations of the present investigation is the use of destructive methods that involve the sectioning of the teeth. However, destruction of evidence may not be permitted by our legal system.[18] The findings in the present study can only be useful in the context of crime investigations, where determination of age was a key factor in the identification of the unknown remains. Further studies could be warranted to study the secondary changes i.e. secondary dentine deposition in the radiographs, dentine translucency in extracted and unsectioned teeth for predicting the attainment of age thresholds of importance in adults.

CONCLUSIONS

In criminal investigations, forensic experts are often requested by the police to provide the age range of the unknown human remains that may help them in proceeding with further investigation. In this study, two popularly used parameters in adult age estimation i.e, the RDT and CW for indicating the age over 55 years were investigated and it was found that there is significant influence of both parameters on the age of the subject. Their thickness increased with age that was statistically significant in both sexes. Both parameters also have exhibited very high probability (approximately 80%) in predicting the age over 55 years. However, factors like need for the extraction of the teeth, sectioning of the teeth and procedural difficulties make age assessment using these parameters less reliable. Moreover, they cannot be applied to living individuals who need age estimation in civil proceedings. Hence, there is a need to find alternate parameters such as study of secondary dentine deposition in radiographs for predicting age thresholds in adults.

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