

Correlation Between Cementum Incremental Lines and Chronological Age of Human Teeth in a South Indian Population: A Forensic Study

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Abstract

Background

Accurate age estimation is an essential component of forensic identification. Cementum exhibits continuous appositional growth throughout life, producing incremental lines that may serve as a reliable biological marker for estimating chronological age. The aim of the study was to evaluate the correlation between cementum incremental line (CIL) counts and chronological age (CA) in human teeth and assess the accuracy of cementum annulation as a forensic age estimation method. The study was conducted on 62 extracted permanent human teeth obtained following orthodontic treatment or trauma. Teeth with caries, periodontal disease, deciduous dentition, or cementum-associated pathology were excluded. Ground cross-sections were prepared from the middle third of the root and examined under a phase contrast microscope at $\times 20$ magnification. The number of cementum incremental lines was determined by dividing the total cementum thickness by the width of two adjacent incremental lines. Estimated age (EA) was calculated by adding the eruption age of the respective tooth to the incremental line count. Statistical analysis was performed using SPSS version 20.0. Paired *t*-test, Pearson's correlation coefficient, and Bland–Altman analysis was used, with statistical significance set at $p < 0.05$.

Results

The mean chronological age was **35.97 ± 12.42 years**, whereas the mean estimated age was **30.27 ± 10.09 years**, with a statistically significant mean difference of **5.70 years** ($p < 0.001$). A strong positive correlation was observed between chronological and estimated age ($r = 0.73$, $p < 0.001$). Premolars ($r = 0.814$, $p < 0.001$) and molars ($r = 0.717$, $p < 0.001$) demonstrated the highest correlations. Bland–Altman analysis showed a mean bias of **6.38 years** with limits of agreement ranging from **– 10.33 to 23.09 years**, indicating moderate agreement and a tendency for age estimation error.

Conclusion

Cementum incremental line analysis demonstrated a significant positive correlation with chronological age, supporting its utility as an adjunctive method for forensic age estimation. However, the variability observed between estimated and actual age indicates that cementum annulation should be interpreted cautiously and used alongside other forensic age estimation techniques to improve accuracy.

INTRODUCTION

Estimation of age is a multifaceted approach that involves skeletal, dental, histological, and biochemical methods to estimate the age of the living and the diseased (Adserias-Garriga, J. 2019). In some locations, age determination can be used as an adjuvant tool where people are uneducated and unaware

of their age (Swetha G et al 2018). Age estimation plays a crucial role in identifying of unknown corpses and skeletal remains of accidents, crimes, and disaster victims (Verma M et al 2019).

There are various methods for dental age estimation like radiographical, histological, odontometric, and biochemical methods (Adserias-Garriga, J. 2019). Once the entire complement of teeth is in place, changes in the dental hard tissues must be relied upon for the age estimation (Kasetty, S. et al 2010). When subjects undergo extensive changes to their external features, it does not gain any information. In such cases, the tooth is used for the identification. The Decay and degradation process is resisted by the hard tissues of the teeth, even after the tissues have been lost (Aggarwal P. et al 2008).

The Cementum is a vascular dental structure. It surrounds the anatomical roots of the human tooth and has a mineralized substance that is continuously deposited on the root surface (Swetha G et al 2018). The sharpness and clarity of the annulations are the most noticeable features of the cementum (Stott G. G et al 1982). cemental apposition has a straight-line relationship with age because its thickness increases continuously with age (Zander, H. A et al 1958). The cemental apposition may be influenced by function, occlusion, and pathologic processes. (Solheim T, 1990). This helps in the approximation of age in forensic investigations. Cementum surrounds the root of the tooth in a layered fashion resulting in a concentric appearance called salter lines which can be used for age estimation (Gupta P et al 2014). One incremental line is formed by a pair of dark and light bands (Kagerer P & Grupe, G, 2001).

Counting alternative white and dark bands of the incremental lines of cementum in addition to the actual age at which the tooth erupts gives an estimation of age in an individual (Swetha G et al 2018). Acellular cementum is considered for counting than cellular cementum since cellular functions in repair and regeneration, but acellular has the lifelong deposition with anchorage function. To evaluate the correlation between cementum incremental line counts and chronological age in human teeth and assess the accuracy of cementum annulation as a forensic age estimation method.

MATERIALS AND METHODS

A cross-sectional study with a sample size of 62 natural teeth was carried out after due approval from Institutional Ethics Committee. Written informed consent for the use of extracted teeth for research purposes was obtained from the participants prior to inclusion in the study. To determine the number of natural teeth to be used in the study, the sample size was calculated.

Teeth extracted for orthodontic treatment or trauma were included, whereas teeth extracted for dental caries, periodontal disorders, deciduous teeth, and teeth with cementum associated diseases were excluded.

Cross-sections of the teeth were prepared at the middle third of the root and examined under a phase contrast microscope with the magnification of 20x. In each section, the middle third of the root was considered for counting since acellular cementum is predominant in this part of the section.

From the cemento-dentinal junction to the surface of the cementum, the thickness was measured, and the width of the two incremental lines, which is most easily recognizable was measured (Fig. 1: Incremental lines of cementum observed using phase-contrast microscopy at ×20 magnification)

The number of incremental lines was calculated by the formula (Aggarwal P. et al 2008).,

Number of incremental lines(n) = X/Y

Where,

X= total width of the cementum,

Y= width of the two incremental lines of the cementum

The estimated age of the teeth was calculated by the formula that is given by Scott.^[7]

Estimated age= Number of incremental lines + Eruption age of that particular tooth

STATISTICAL ANALYSIS:

Statistical analyses were performed using Statistical Software Package for the Social Sciences (SPSS) 20.0 version. Comparison of actual age and estimated age was made using paired t-test. Correlation between the actual age and estimated age was done using Karl Pearson’s correlation.

RESULTS

Comparison of age and estimated age were performed using paired t-test. The mean and standard deviation of the actual age is 35.97± 12.42. The mean and standard deviation of the estimated age was found to be 30.27 ± 10.09. Comparing the mean of actual age and estimated age, the mean value of actual age is higher than the estimated age with a mean difference of 5.7016 with a statistically significant p-value of <0.001(table 1).

Table 1 Comparison of actual age and estimated age using paired t-test

		Mean ± SD	Mean difference ± SD	t	P-VALUE
Pair 1	AGE	35.97±12.42	5.7±8.55	5.25	<0.001
	ESTIMATED AGE	30.27±10.09			

Correlation between the actual age and the estimated age was performed using Karl Pearson’s correlation, and the results showed a very good positive correlation between the actual age and the

estimated age with a statistically significant p-value of <0.001(table 2). The results revealed that when the actual age of the individual increased, the number of incremental lines increased significantly.

Table 2 Correlation between actual age and estimated age using Karl Pearson's correlation

SNO	PARAMETERS BEING CORRELATED	N	Correlation(r)	P-VALUE
1	AGE & ESTIMATED AGE	62	0.73	<0.001

Further interpreting by the type of tooth,

Incisor: The mean value of actual age is higher than the estimated age with the mean difference of 8 with the statistically significant p-value of 0.008 and showed a very good positive correlation but with a not significant p-value of 0.068 (table3, 4).

Table 3 Based on the type of tooth, comparison of actual age and estimated age using paired t-test

			N	Mean $\pm$ SD	Mean difference $\pm$ SD	t	P-VALUE
Incisor	Pair 1	AGE	9	39.44 $\pm$ 8.6	8 $\pm$ 6.78	3.54	0.008
		ESTIMATED AGE	9	31.44 $\pm$ 6.64			
Canine	Pair 1	AGE	4	34.25 $\pm$ 10.28	0.5 $\pm$ 6.46	0.16	0.887
		ESTIMATED AGE	4	33.75 $\pm$ 5.68			
Premolar	Pair 1	AGE	26	34.5 $\pm$ 14.65	3.83 $\pm$ 8.72	2.24	0.034
		ESTIMATED AGE	26	30.67 $\pm$ 10.06			
Molar	Pair 1	AGE	23	36.57 $\pm$ 11.57	7.83 $\pm$ 8.82	4.25	<0.001
		ESTIMATED AGE	23	28.74 $\pm$ 11.89			

Table 4 Based on the type of tooth, the correlation between actual age and estimated age using Karl Pearson's correlation

	SNO	PARAMETERS BEING CORRELATED	N	Correlation(r)	P-VALUE
Incisor	1	AGE & ESTIMATED AGE	9	0.631	0.068
Canine	2	AGE & ESTIMATED AGE	4	0.824	0.176
Premolar	3	AGE & ESTIMATED AGE	26	0.814	<0.001
Molar	4	AGE & ESTIMATED AGE	23	0.717	<0.001

Canine: The mean value of actual age is higher than the estimated age with the mean difference of 0.5 which is not statistically significant p-value of 0.887 and showed an excellent positive correlation but with a not significant p-value of 0.176.

Premolar: The mean value of actual age is higher than the estimated age with the mean difference of 3.8269 with the statistically significant p-value of 0.034 and showed an excellent positive correlation with a significant p-value of <0.001.

Molar: The mean value of actual age is higher than the estimated age with the mean difference of 7.8261 with the statistically significant p-value of <0.001 and showed a very good positive correlation with a significant p-value of <0.001.

Table 5 Bland-Altman Analysis

Bland-Altman			
		95% Confidence Interval	
	Estimate	Lower	Upper
Bias (n = 62)	6.38	4.21	8.54
Lower limit of agreement	-10.33	-14.05	-6.61
Upper limit of agreement	23.09	19.37	26.81

The agreement between the two methods assessed using Bland–Altman analysis (n = 62) demonstrated a mean bias of 6.38 years (95% CI: 4.21 to 8.54), indicating that the cementum annulations method tends to overestimate age compared to the reference method. The limits of agreement ranged from –10.33 to 23.09 years, with the lower limit of agreement at –10.33 (95% CI: –14.05 to –6.61) and the upper limit at 23.09 (95% CI: 19.37 to 26.81). This wide range of limits suggests considerable variability between the two methods, indicating moderate agreement with a tendency toward overestimation by cementum annulations.

DISCUSSION

Age estimation plays a crucial role in the identification of unknown corpses and skeletal remains of accidents, crimes, and disaster victims.^[4] There are various methods for dental age estimation like radiographical, histological, odontometric and biochemical methods (Adseries-Garriga, J., 2019).

When subjects undergo extensive changes to their external features, which yield no information, the only means of identification used is their teeth. Also, without any gross changes human teeth can be preserved for over a long period of time (Gaballah O et al, 2015). Even after the tissues have been lost, the human dentition's hard tissues can resist decay and degradation. This resistance of the teeth has

made it a helpful indicator at the time of disease for calculation of age, assessing variation in diet, expression of metabolic diseases (Aggarwal P et al, 2008).

The basis for the age estimation is the gradual structural changes in the teeth throughout life. The enamel, dentin, and cementum have been used to estimate the chronologic age of unidentified individuals (Vaidya S et al, 2015).

In humans, cementum grows progressively throughout life. Tooth cementum is the calcified ectomesenchyme tissue that surrounds the dentin (Verma, M et al, 2019). Microscopically, cementum appear as a series of alternating light and dark lines or bands that have the appearance of tree annual rings (Gaballah O et al, 2015).

Acellular cementum undergoes appositional growth that results in alternating layers of dark and light bands which can be seen in microscopic sections (Kagerer P & Grupe, G, 2001). A pair of dark and light bands constitute an incremental line. It has been found that cementum growth is approximately linear and cementum thickness is proportional to the age of the tooth (Sousa E. M et al, 1999). Zander, H. A., & Hurzeler, B. (1958) stated that cementum is potentially a better age-estimating tissue due to its unique location in the alveolar process. (Stott G. G et al 1982) were the first to test this method.

In the current study, incisors, canines, premolars, and molars were used for the analysis. Similar to our study, Jankauskas et al typically used incisors, canines, premolars, and molars to count cemental lines (Jankauskas R et al, 2001). Dias et al, 2010 and Aggarwal et al, 2008 also studied all the maxillary and mandibular teeth.

In the current study, ground sections of the teeth were used for age estimation. In literature, there has been no unanimity in the sectioning method. Some authors prefer sections to be cross-section, whereas others prefer longitudinal. Stott et al. in their studies also preferred cross-sections that allowed a series of observations (Stott G. G et al 1982). Aggarwal et al. (2008) in their studies used longitudinal ground sections.

In the present study we have utilised 62 cross-sections of teeth. For counting of cemental lines, the middle third of the root was considered because the cementum that is usually present in this region is acellular, which is undisturbed and even in growth and such annulations can be counted easily without any hindrance (Kaur P et al, 2015).

The ground sections were examined under a phase contrast microscope under 20x magnification with eyepiece micrometry. Previously, observers in the study either had to count directly while looking through the microscope or use simple photographs. Features crucial to the improvement of results include using various microscopic methods, like polarizing and phase-contrast microscopy and digital image enhancement procedures (Agrawal, G., 2010).

In the current study, comparing the mean of the actual age and estimated age gives the statistically significant p-value of < 0.001 . While Swetha G et al, 2018 in their study by comparing mean, showed a

statistically not significant observation with the p-value of ≥ 0.05 . Our study results also showed a very good positive correlation between the actual age and estimated age. While Kaur P et al., 2015 showed an excellent positive correlation and Swetha G et al, 2018 in their study also showed a positive correlation.

In the current study, the derived correlation of actual age and estimated age was $r = 0.73$. A study by Stein, T. J., & Corcoran, J. F., 1994 gave a much higher correlation between predicted age and known age ($r = 0.93$). Selukar M. S. et al, 2002 found a significant positive correlation between the age of individual and cementum apposition and concluded that when age advances, the cemental apposition and number of incremental lines are increased. In our study also, we have observed that, when age increases, the number of incremental lines of the cementum were increased.

Currently in our study, based on the type of tooth, the results were shown. Mean comparison values of actual age and estimated age in incisor, premolar and molar showed a statistically significant p-value and correlation between the actual age and estimated age parameters in premolar and molar showed a statistically significant p-value of < 0.001 , where premolar showed excellent correlation.

The result of the current study demonstrates a very good positive correlation between the individual's actual age and estimated age.

CONCLUSION

In forensic medicine, the calculation of the cemental line of the tooth can be used to estimate age. The results revealed a significant correlation in estimating the individual's actual age. This also demonstrated that as an individual's age increases, the number of cemental lines increases significantly. This suggests that cementum deposition occurs over the course of one's life and can be used to determine one's age.

However, in the cross section of the tooth, the cemental thickness varies. Because of the variable thickness of the cementum, an inaccuracy in estimating age may occur while counting them.

Abbreviations

CA

Chronological Age

CIL

Cementum Incremental Lines

EA

Estimated Age

n

Sample Size

p

Probability Value

r

Pearson's Correlation Coefficient

SD

Standard Deviation

SPSS

Statistical Package for the Social Sciences

×

Magnification

Declarations

Author Contribution

• Makesh Raj L.S – Conceptualization, Formal analysis, Investigation, Methodology, Writing • S.Prasanna - Formal analysis, Investigation, Methodology, Writing • Srikant N – Validation, Data curation, Review & editing • Uzma Iqbal Belgaumi - Review & editing

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Figures

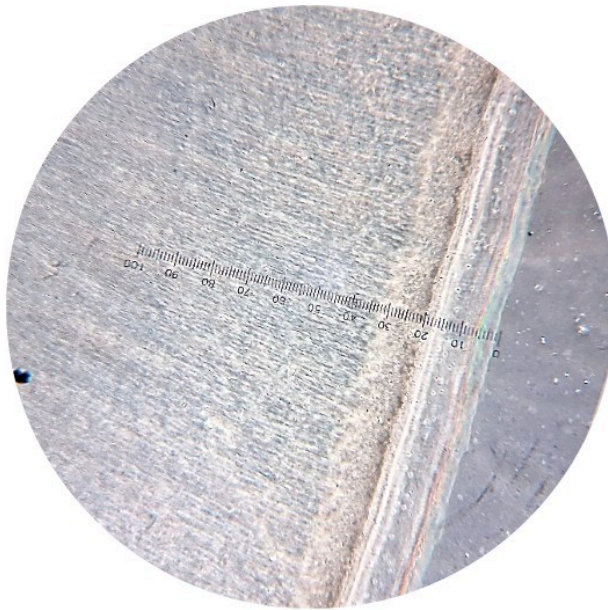


Figure 1: Incremental lines of cementum using phase contrast microscope with magnification of 20x

Figure 1

Incremental lines of cementum observed using phase-contrast microscopy at $\times 20$ magnification.

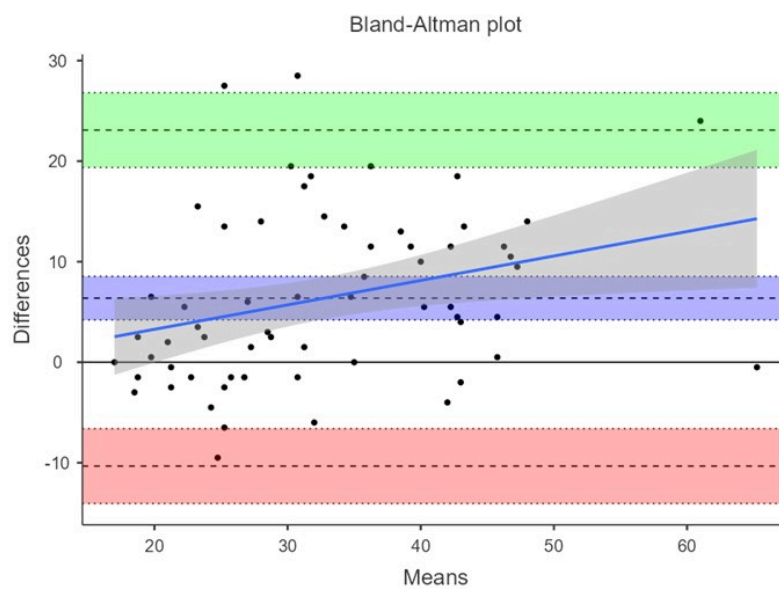


Figure 2 : Bland-Altman Analysis

Figure 2

Bland–Altman Analysis of Chronological Age and Estimated Age

