

# Age estimation by cemental annulation rings

N. Mohan,  
Sabitha Gokulraj,  
Merlyn Thomas  
*Department of Oral Medicine and  
Radiology, Vinayaka Missions  
Sankarachariyar Dental College,  
Salem, Tamil Nadu, India*

**Address for correspondence:**  
*Dr. Merlyn Thomas,  
2/B 301, Om Shivalaya,  
Eden Rose, Opp. Cine Max,  
Beverly Park, Mira Road (E),  
Mumbai - 401 107, Maharashtra,  
India.  
E-mail: merlynthomas89@  
gmail.com*

## Introduction


According to Keiser-Neilson in 1970, forensic odontology is a branch of medicine which, in the interest of justice, deals with the proper handling and examination of dental evidence and with proper evaluation and presentation of dental findings.<sup>[1]</sup> Age is one of the essential factors in establishing the identity of a person, and age estimation is a method which is adopted by anthropologists, archaeologists, and forensic scientists.<sup>[2]</sup> Teeth differ from bones in their biological properties and function and

## Abstract

**Context:** Tooth plays a pivotal role in the identification of a person when all other remains are ruined by natural or unnatural causes. Dental evidence can contribute to age estimation in forensic dentistry. Estimating the age of an individual with the help of a tooth for identification proves beneficial. Alternating light and dark bands seen on the cementum have been shown to be proportionate to the age of the patient in number. This was done to evaluate the cementum annulation in age determination. **Aims:** This study aimed to determine the age of an individual using tooth cementum annulation. **Materials and Methods:** In this study, twenty extracted teeth were collected and all the clinical details of the patient such as age, sex, and chief complaint were recorded. The collected teeth were preserved in formalin overnight and then washed under tap water before they were sectioned. The area selected for counting was observed under 10 × objective of light microscope, and photomicrographs were taken for the counting of the lines. At the time of analysis, the age of the patient was not disclosed to the observer. **Statistical Analysis Used:** Student's *t*-test was used for statistical analysis. **Results:** This study showed the correlation between the actual age of the individual and the estimated age using tooth cementum annulation method and showed significant statistical value. **Conclusion:** Incremental lines found in cementum can be used for age estimation in forensic dentistry.

**Key words:** Age estimation, tooth cementum annulation, forensic odontology

are preserved well; thus teeth are important tools for identification of age of an individual. Forensic dentists are the major contributors in age estimation. Cementum, which covers the anatomical root, remains stable throughout an individual's life as the racemization reaction of aspartic acid in cementum continues in a constant manner.<sup>[3]</sup> Tooth cementum annulation method has gained equal success even in archaeological preservation. The purpose of this study is to estimate age using tooth cementum annulation method by counting the incremental lines present in the cementum.

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## Materials and Methods

This is a prospective study performed after obtaining institutional ethical clearance and carried out for 3 weeks; the limited sample size of this study was due to short time duration. The study consisted of twenty freshly extracted teeth from patients aged 15–39 years. Cases were selected depending on the availability and the sample size was fixed and hence there was no randomization. The sample used in this study was extracted premolars and molars from both the upper and lower jaws. The selected teeth were extracted for orthodontic purpose or due to carious involvement. Periodontally compromised teeth and teeth subjected to attrition, abrasion, and erosion were not included in the study. Patients subjected for orthodontic extraction and extraction due to caries without periodontal involvement were included in the study. Patient selection bias was avoided by including the sample based only on the inclusion criteria. The extracted teeth were collected and stored overnight in 10% formalin and then thoroughly washed under running water before they were grounded to obtain thin section of 80 to 120  $\mu\text{m}$  thickness. Sectioning was done longitudinally and hence the incremental lines were parallel to the long axis of the tooth. After sectioning, the specimens were again washed under running water and immersed in xylene to clear off all the debris from the sectioned specimen. The sections were then mounted on a glass slide with DPX mountant and allowed to dry for 4 h. The slide was later viewed under light microscope with a 10 $\times$  objective. Photomicrographs of each prepared slide were taken and recorded and areas where incremental lines were continuous and easy to count irrespective of whether cellular or acellular cementum was considered. Photomicrographs were taken by using Cannon IXUS 190, Malaysia, digital camera and the lens was magnified five times. The photographs were further processed with Adobe Photoshop CS6 software, and counting was done on enlarged photographs by two separate observers. The observers were blinded to the age of the individual and the average of the result was obtained. The age of the patient was thus obtained by adding the number of counted incremental lines to the age of the eruption of the tooth as follows:

Age of the patient = Number of incremental lines + the age of the tooth eruption.

### Statistical analysis

Statistical analysis was done using regression slope, correlation table, and Student's *t*-test.

## Results

In this study, twenty extracted teeth were sectioned for counting incremental lines in cementum [Figures 1 and 2]. Both cellular and acellular cementums were viewed for counting

the lines. Dark and light incremental lines of cementum were not clearly viewed in all the sections. Only the dark lines suitable for counting were selected. The age of the individual was obtained by adding the total number of incremental lines [Figure 3] from each slide to the eruption age of that tooth [Table 1 and Figure 4]. Table 2 and Figure 5 represents the frequency of age in years where age 15 had a frequency of 1 which made up to 5% of the total

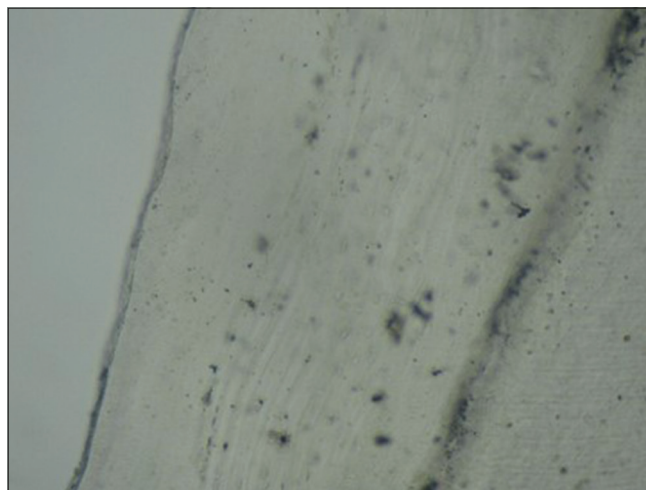


Figure 1: Cellular cementum showing cemental bands under  $\times 10$

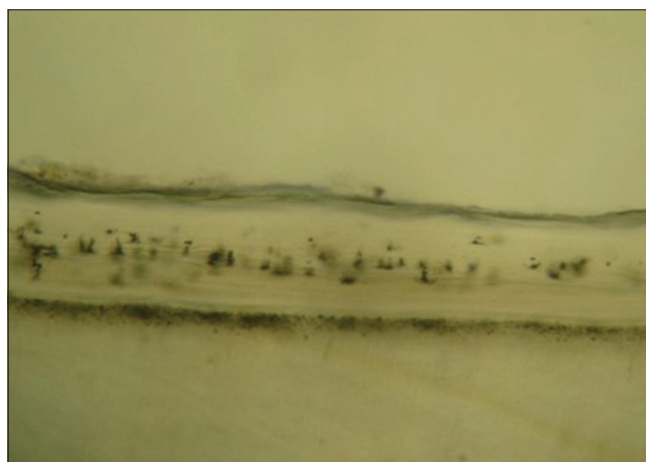


Figure 2: Cellular cementum showing cemental bands under  $\times 10$

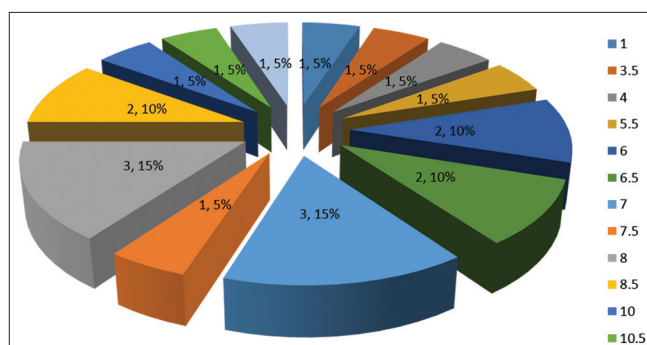


Figure 3: PI diagram representing frequency of mean incremental lines

**Table 1: Frequency of age of the tooth eruption**

Age of the tooth eruption	Frequency (%)
10.5	11 (55)
11.0	5 (25)
11.5	1 (5)
19.0	3 (15)
Total	20 (100)

**Table 2: Frequency of age in years**

Age (years)	Frequency (%)
15	1 (5)
16	4 (20)
17	2 (10)
18	3 (15)
19	2 (10)
20	1 (5)
21	2 (10)
22	1 (5)
23	1 (5)
24	1 (5)
26	1 (5)
39	1 (5)
Total	20 (100)

number of teeth and so on. Similarly, Table 3 represents the frequency of mean incremental lines where the mean incremental line of 1 had an occurrence of 5% and so forth. Frequency of the age of the tooth eruption is signified in Table 1, for example, dental age of 10.5 had a frequency of 55%. Data obtained by using the regression equation in which the mean incremental lines and the dental age correspond to the actual age of the patient are signified in Table 4. Comparison was made statistically between the estimated and chronological ages using a linear regression slope [Figure 6]. Using the regression equation, the age is predicted corresponding to the mean incremental lines and age of the tooth eruption [Table 4 and Figure 6]. Considering the significance values, we observe that mean incremental lines and age of the tooth eruption are significant ( $P < 0.01$ ) in predicting the ages [Tables 5 and 6]. Hence, no significant difference was observed over the observed and predicted ages. The predicted age based on the cemental lines observed in the present study matched with the results of the previous studies.

## Discussion

Age estimation in forensic science can help the investigator in identifying a person during a crime or a mass disaster. Various methods in age identification include assessing dental or skeletal morphology and laboratory methods such as racemization of aspartic acid in dentin or tooth enamel or radiocarbon dating of tooth enamel.<sup>[4]</sup> In age estimation, the role of a tooth comes into play when all other remains of a human are destroyed. Teeth are well preserved in heat and

**Table 3: Frequency of mean incremental lines**

Mean incremental lines	Frequency (%)
1.0	1 (5)
3.5	1 (5)
4.0	1 (5)
5.5	1 (5)
6.0	2 (10)
6.5	2 (10)
7.0	3 (15)
7.5	1 (5)
8.0	3 (15)
8.5	2 (10)
10.0	1 (5)
10.5	1 (5)
25.0	1 (5)
Total	20 (100)

**Table 4: Age prediction using regression equation corresponding to mean incremental lines and age of tooth eruption**

Observed age	Mean incremental lines	Age of the tooth eruption	Predicted age
19	8	10.5	19.06
21	10	10.5	21.22
26	8.5	19	27.29
21	10.5	11	22.21
39	25	11.5	38.29
23	4	19	22.44
16	6	11	17.36
18	6.5	10.5	17.45
16	7.5	10.5	18.52
15	5.5	10.5	16.37
18	7	10.5	17.98
16	3.5	11	14.66
19	8.5	11	20.05
17	7	10.5	17.98
16	6.5	10.5	17.45
22	7	10.5	17.98
18	8	10.5	19.06
17	6	11	17.36
20	1	19	19.2
24	8	10.5	19.06

mass disasters and thus the tooth remains prove beneficial in investigations.

Root dentin surface consists of a bone-like mineralized tissue known as cementum which is secreted by cementoblasts. When the epithelial cells of Hertwig's root sheath and mesenchymal cells of the dental follicle come in proximity with the developing root surface, the cementum formation begins, though the biology behind the contribution of epithelial and mesenchymal components toward cementogenesis is subtle. The established theory states that after passing the barrier of Hertwig's epithelial root sheath, mesenchymal cells of the dental follicle turn into cementoblasts and secrete cementum.<sup>[5]</sup>

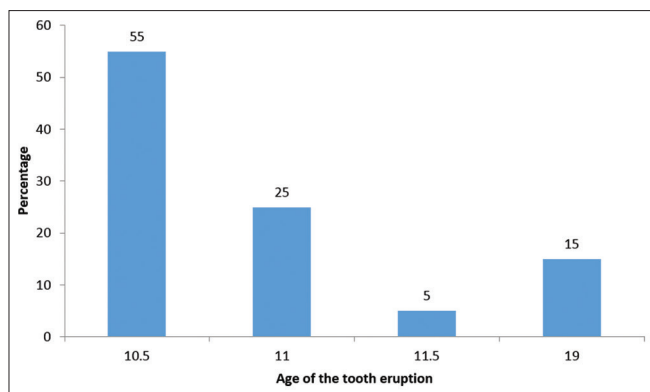


Figure 4: Bar diagram representing age of the tooth eruption

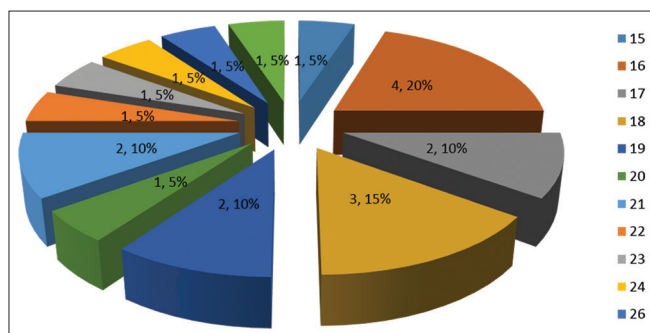


Figure 5: PI diagram representing frequency of age in years

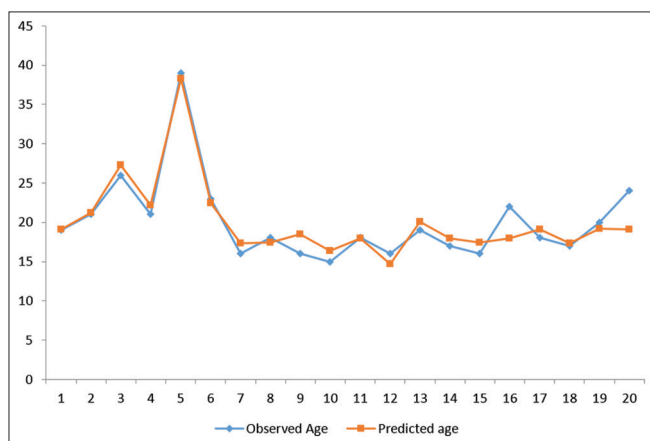


Figure 6: Regression slope plotting the observed age and predicted ages

Liebermann<sup>[6]</sup> has stated that there is continuous deposition of cementum throughout the life of an individual unlike the other dental tissues. Studies have been performed previously to determine the accuracy of tooth cementum annulation method in age estimation.<sup>[7]</sup> Remodeling or resorption of cementum takes place rarely because it is avascular.

Incremental lines are present in both cellular and acellular cementums and the bands in acellular cementum grow at a slow and constant rate as compared to that in cellular cementum.<sup>[6]</sup> Natural metabolic rhythm caused by seasonal

Table 5: Correlation table showing significant correlation between age and dependent variables of mean incremental lines except age of the tooth eruption

	Age (years)	
	Pearson's correlation	P
Mean incremental lines	0.799	<0.001**
Age of the tooth eruption	0.284	0.112

\*\*Highly significant

Table 6: Mean and standard deviation for each variable in the analysis

	n	Mean	SD	R <sup>2</sup>
Age	20	20.05	5.38	0.886**
Mean incremental lines	20	7.700	4.62	
Age of the tooth eruption	20	11.950	3.05	

\*\*Highly significant. SD: Standard deviation

changes results in layering of the cementum bands. Parathyroid hormones which, in association with Vitamin D, regulate the calcium absorption play an important role in the incremental line formation.<sup>[3]</sup> Intercemental lines represent a yearly incremental deposition of cementum and these lines are counted for estimating age in animals.<sup>[7]</sup>

Pundir *et al.* studied age by counting the incremental lines with the section thickness of 80 μm and were examined under light microscope, polarized microscope, and phase-contrast microscope. Staining of longitudinal sectioning from teeth preserved in formalin can be done using various techniques and incremental lines in cementum can be observed in conventional light microscopy, but it does not give a satisfactory result. Since acids and stains do not destroy the incremental lines, it is likely that they contain organic structure which is different from that of the cementum.<sup>[7]</sup>

Condon *et al.* in their study showed a high correlation between adjusted counts of cementum layers (i.e., the number of layers added to the age of tooth eruption) and age at extraction or death.<sup>[8]</sup>

Many researchers have used cemental annulations to determine the age of adults, but at present there is controversy in using this method because different studies show vast discrepancies in the results.<sup>[9]</sup>

Mallar *et al.* concluded that annulations are most appropriately counted in the middle third of the tooth root and, though the cross sections are easier to count, more reliable results are determined from longitudinal sections.<sup>[10]</sup>

Kaur *et al.* determined that incremental lines of cementum were most clearly visible under a phase-contrast microscope and subsequently under polarized microscope and light microscope.<sup>[11]</sup> Analysts determined the observer error and tooth variability in this technique for age estimation and concluded that premolars are the preferred tooth with

least interobserver error. Also, by increasing the number of slides per tooth, tooth variance can be minimized.<sup>[12]</sup>

Radović used cemental annulations ring method to determine age of Djerdap anthropological series, dated to a period from the 10<sup>th</sup> to the 6<sup>th</sup> millennium BC. On several teeth, taphonomy changes of tooth cementum were noticed which hindered the complete application of the tooth cementum annulation method.<sup>[13]</sup>

Avadhani *et al.* instituted that the reliability of the method was 94.73% as the determined age in their study varied by 2-3 years with the actual age.<sup>[14]</sup>

In this study, age estimation using tooth cementum annulation method under light microscope showed a significant correlation between the observed age and estimated age of the individual.

## Conclusion

Tooth cementum annulation method can be used in forensic odontology for estimating the age of an individual. This is a pilot study and larger samples are required to determine the accuracy in age estimation using light microscope, in contrast to many other established studies determining the incremental lines using light, polarized, and phase-contrast microscopes. Many studies have shown that the number of incremental lines in cementum remains constant even in cremated or inhumed species, and only the width and degree of mineralization vary. Application of this method with accurate results in periodontally compromised teeth has not been proven. Cementum annulation method can be applied to evaluate age even in cases of poor preservation. Several studies have also shown that cementum annulation method can be used in determining age in anthropological study of ancient population. More studies comparing the different methods for studying the cementum annulation can establish the usefulness of this method in age estimation.

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## Conflicts of interest

There are no conflicts of interest.

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