

Cemental annulation: An approach to age estimation in transverse and longitudinal sections using three different microscopes

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Abstract

Background: Estimation of age is an important aspect in determining a person's identity. Since teeth are resistant to decay and degradation, estimation of age using dental structures may be a valuable tool in human identification. Recent research suggests that tooth cemental annulation could be used as an effective method for age estimation than other morphologic or histological parameters.

Materials and Methods: A total of 100 extracted teeth were chosen. Using a hard tissue microtome (SP 1600) longitudinal and transverse ground sections of 100 µm thickness of each tooth were prepared. It is further examined under light microscopy, polarized microscopy and phase-contrast microscopy. The images of longitudinal and transverse were magnified on a computer and the cemental lines were counted.

Results: The present study showed is a strong positive correlation for cemental annulation between the calculated age and estimated age when phase-contrast microscopy was used in both longitudinal and transverse sections when compared with other microscopes. The correlation coefficient value was found more significant in the longitudinal section which implies that the phase-contrast microscope was highly reliable and had a significant correlation ($P < 0.001$) with the actual age compared to other microscopes.

Conclusion: Cemental annulation by various microscopes improves accuracy in the prediction of age and also it allows for age estimation in cases where skeletal fragments are poorly preserved.

Keywords: Age estimation, cemental annulation, light microscopy, phase-contrast microscopy, polarized microscopy

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INTRODUCTION

Age is one of the most important factors that affect every aspect of life. The estimation of age using dental structures may be of great help in human identification. It may also

be useful in other situations, like determining the legal liability of teenagers and adults of unknown age, as well as assisting with research.^[1]

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A cornerstone of forensic science is the identification of individuals by using the unique characteristics and traits of the teeth and jaws.^[2] Age determination is critical in forensic medicine, not only in the identification of bodies but also in the investigation of crimes. When the external characteristics no longer provide sufficient information, the teeth could be a possible means of identification.^[3]

Human dentition can withstand decay and degradation for a long duration which could be used as an indicator for assessing variation in diet, expressing metabolic diseases and calculating age. For forensic investigators dealing with unknown bodies, an accurate method estimation of age is critical from human skeletal tissue, which is still unknown.^[4]

Dental age estimation is based on changes that occur during tooth formation and eruption or on a continuous process that alters and degrades the quality of dental tissues even after growth is completed.^[5] Tooth cementum annulations (TCA) are a microscopic method for determining an individual's age based on an examination of acellular extrinsic fiber cementum.^[6]

Cementum deposition continues throughout life, particularly during the stress response. In humans, for example, as the tooth crown wears down, new cementum is deposited on the roots, allowing the tooth to gradually rise higher in the socket while maintaining good occlusion.^[7, 8] Numerous studies have been done, wherein tooth cemental annulations have been used as a criterion for estimation of age in both land and sea animals.^[9]

Over the past 30 years, researchers have used cemental annulations as a reliable tool to determine age.^[10] According to Zander and Hurzeler, cementum is considered to be a better age estimating structure because of its unique location in the alveolar process. Based on biological factors in the formation of the tooth cemental annulations, the hypothesis that these incremental lines are more reliable age marker.^[11]

In humans, a positive association between cemental annulations and age determination has been reported by Stott *et al.*, however, according to research by Lipsinic *et al.* and Miller *et al.*, cemental annulations cannot be used as a reliable tool for age identification.^[12] Hence the studies about age estimation using cemental annulation have produced an inconsistent and obscure result.

Considering the above facts, the present study was designed with the following objectives (1) to assess the effectiveness cemental annulation method in age

estimation in the human population and (2) to correlate age estimation in transverse and longitudinal sections using three different microscopes.

MATERIALS AND METHODS

A sample size of 100 extracted teeth was taken up for the study. The patient case records were studied and the details were noted in the proforma to exclude pathologies such as attrition and root caries and considered sound tooth which was extracted for orthodontic treatment and each tooth was stored in 10% formalin in individual boxes [Figure 1].

Method

Using a hard tissue microtome (SP 1600), 50 longitudinal and 50 transverse ground sections of 100 μ m thickness of each tooth was prepared [Figure 1]. Incremental lines of cementum from the prepared longitudinal and cross-sections were then studied using a light microscope, phase-contrast and polarized microscope. Photomicrographs were taken focusing on the cementum of the middle third region of the root using a digital camera OLYMPUS U-CMAD3 under $\times 10$ magnification and were then transmitted from the research microscope (BX41) to a monitor.

Each dark band along with the light band following it constituted one annulation. Teeth with indistinct, invisible cemental lines were eliminated. The mid-root section was chosen for counting the annulations for the following reasons:^[13]

- The thickness, width and cellularity of the cementum layers and also the number of resorption areas increase apically, complicating annulation counting
- The thinness of the cementum near the tooth's neck prevents scoring



Figure 1: DPX mounted transverse and longitudinal ground section on the slides

- To reduce the influence of factors known to obscure annulations or cause cementum variation, such as periodontal disease and hypercementosis caused by local or systemic disease.

Cementum lines were counted manually by marking a point against each line observed and then counting the number of points marked in total per photomicrograph. For each alternating light and dark band of cementum annulations, a score of 1 was noted and the total number of incremental lines was counted.

Chronological age was estimated using the formula:^[14]

$$\text{Estimated Age} = \text{Total number of cementum annulations} + \text{Age of eruption of that tooth}$$

The number obtained was then compared with the known age of subjects. A similar procedure was followed using the polarizing microscope and phase-contrast microscope and the same comparative analysis was done [Figures 2-4].

Statistical analysis

The photographs were evaluated by 2 independent observers who are trained to evaluate incremental lines as described by Mallar KB *et al.*,^[13] Each observation was tabulated independently and also each observation by observers was subjected to Cohens weighted Kappa analysis. The number of incremental lines was counted, and the number was entered in the excel sheet. The present study indicated a substantial agreement of 0.6 between the 2 observers, any discrepancies between the finding were solved by active discussion, collected data were subjected to descriptive statistics using IBM SPSS Statistics, Version 22.0 (Armonk, NY, USA). Comparison of actual age and estimated age was done by paired *t*-test whereas Intragroup correlations were carried out by Karl Pearson's correlation coefficient.

RESULTS

The mean age of annulations in cross-section and longitudinal sections have been represented in Table 1. The mean actual age in years is 38.44 ± 17.94 for

cross-section and 46.54 ± 17.05 for longitudinal sections. The mean estimated age in the case of the cross-section is 30.91 ± 14.50 for light microscope, 32.98 ± 15.16 for polarized light microscope and 34.98 ± 15.86 using a phase-contrast microscope. Whereas mean estimated age for the longitudinal section is 38.96 ± 14.53 for light microscope, 41.42 ± 15.23 for polarized light microscope and 44.78 ± 16.32 by using a phase-contrast microscope.

Table 2 shows the Pearson correlation of actual age and estimated age in cross-section and longitudinal sections. The *r*-value of cross-section and longitudinal section comparing actual age and calculated age using the light microscope is $r = 0.993$ and $r = 0.987$, respectively, with $P < 0.01$ which is significant. The *r*-value of cross-section and longitudinal section comparing actual age and calculated age using the polarized microscope shows $r = 0.997$ and $r = 0.990$, respectively, with $P < 0.01$, which is highly significant. Similarly, the *r*-value of cross-section and longitudinal

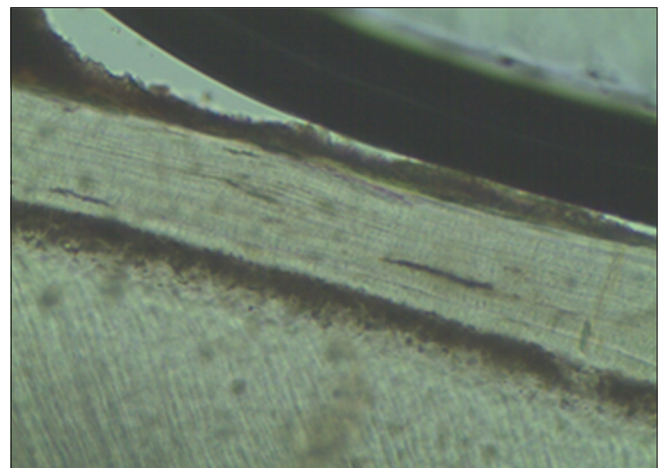


Figure 2: Ground section of the tooth showing cemental lines under a compound microscope

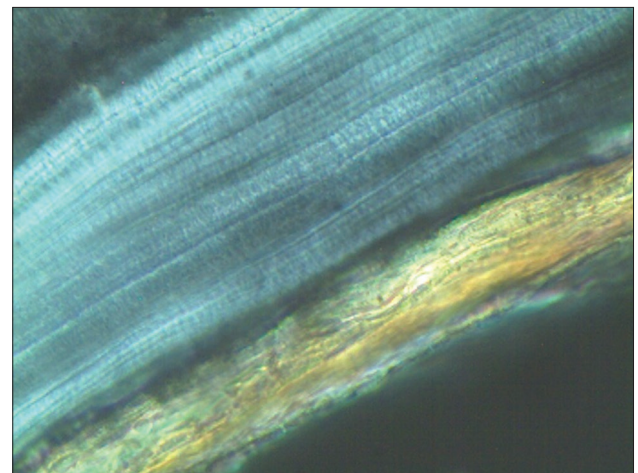


Figure 3: Ground section of the teeth showing cemental lines under a polarizing microscope

Table 1: Mean age of annulations in cross-section and longitudinal section

Annulations	Cross-section	Longitudinal section
Actual age	38.44±17.94	46.54±17.05
ES-LM estimated age	30.91±14.50	38.96±14.53
ES-polarized microscope estimated age	32.98±15.16	41.42±15.23
ES-phase-contrast microscope estimated age	34.98±15.86	44.78±16.32

LM: Light microscope, ES: Estimated age

section comparing actual age and calculated age using the phase-contrast microscope shows $r = 0.998$ and $r = 0.999$, respectively, with $P < 0.01$ which is also highly significant.

Table 3 represent the comparison of actual age and estimated age in various sections. According to the result of the study, there is a statistically significant difference of actual age between cross-section and longitudinal section $P < 0.05$. The actual age of longitudinal is significantly higher as compared to cross-section. Whereas there is a statistically significant difference in estimated age between cross-section and longitudinal section in the case of light microscope 30.91 ± 14.50 and 38.96 ± 14.53 , respectively, with $P < 0.01$. The estimated age by using a polarized light microscope was highly significant for the longitudinal section than the cross-section with 32.98 ± 15.16 and 41.42 ± 15.23 , respectively. Similarly, the phase-contrast microscope estimated age of the longitudinal section is significantly higher as compared to the cross-section 34.98 ± 15.86 and 44.78 ± 16.32 , respectively.

Table 4 represent the comparison of actual age and calculated age in years based on annulation in cross-sections. There is a statistically significant difference of actual age and light microscope estimated age $P < 0.05$. The light Microscope estimated age is not significantly similar to actual age in cross-section with a difference of 7.53 ± 12.32 years. Whereas polarized and phased contrast microscope shows

there is no statistically significant difference of actual age and Pol estimated age $P > 0.05$ with the difference of 5.46 ± 11.41 and 3.46 ± 9.65 , respectively, in cross-section.

Table 5 represent the comparison of actual age and calculated age in years based on annulation in the longitudinal section. There is a statistically significant difference of actual age and estimated age by using a light microscope with $P < 0.05$ showed a difference of 16.3%. which shows estimated age is not significantly similar to actual age in the longitudinal section. Whereas there is no statistically significant difference of actual age and estimated age with polarized light with $P > 0.05$ shows the difference of 11.0%. In the case of a phase-contrast microscope, there is no statistically significant difference between actual age and estimated age $P > 0.05$ which shows a difference of 3.78%.

DISCUSSION

Dental age estimation is a critical component of forensic investigations, with applications ranging from comparing antemortem and postmortem identification to estimating age in children and adults.^[15] According to research, cementum annulation is more reliable in estimating age than skeletal and morphological characteristics.^[16] Cementum is a distinct avascular mineralized tissue that aids in the attachment of periodontal ligament fibers to the root.

Table 2: Pearson correlation of actual age and calculated age

Pairs of variables	Cross-section		Longitudinal section	
	r	P	r	P
Actual age versus calculated age LM	0.993	0.01**	0.987	0.01**
Actual age versus calculated age polarized microscope	0.997	0.01**	0.990	0.01**
Actual age versus calculated age phase-contrast microscope	0.998	0.01**	0.999	0.01**

**Highly significant. LM: Light microscope, ES: Estimated age

Table 3: Comparison of actual age and calculated age in years based on annulations between cross-section and longitudinal section

Annulations	Mean±SD		t-test	P
	Cross-section	Longitudinal section		
Actual age in years	38.44±17.94	46.54±17.05	2.303	0.023*
ES-LM estimated age years	30.91±14.50	38.96±14.53	2.762	0.007**
ES-polarized microscope estimated age years	32.98±15.16	41.42±15.23	2.785	0.006**
ES-phase-contrast microscope estimated age years	34.98±15.86	44.78±16.32	3.029	0.003**

*Significant, **Highly significant. SD: Standard deviation, LM: Light microscope, ES: Estimated age

Table 4: Comparison of actual age and calculated age in years based on annulation in cross-sections

Annulations	Cross-section		t-test	P
	Mean±SD	Difference mean±SD		
Actual age in years	38.44±17.94	7.53±12.32	2.311	0.023*
ES-LM estimated age years	30.91±14.50			
Actual age in years	38.44±17.94	5.46±11.41	1.643	0.104
ES-polarized microscope estimated age years	32.98±15.16			
Actual age in years	38.44±17.94	3.46±9.65	1.022	0.309
ES-phase-contrast microscope estimated age years	34.98±15.86			

*Significant. SD: Standard deviation, LM: Light microscope, ES: Estimated age

Table 5: Comparison of actual age and calculated age in years based on annulation in longitudinal sections

Annulations	Longitudinal section		t-test	P
	Mean±SD	Difference mean±SD (%)		
Actual age in years	46.54±17.05	7.58±12.83 (16.3)	2.368	0.020*
ES-LM estimated age years	38.96±14.53			
Actual age in years	46.54±17.05	5.12±12.02 (11.0)	1.567	0.120
ES-polarized microscope estimated age years	41.42±15.23			
Actual age in years	46.54±17.05	1.76±8.43 (3.78)	0.522	0.603
ES-phase-contrast microscope estimated age years	44.78±16.32			

*Significant. SD: Standard deviation, LM: Light microscope, ES: Estimated age

It is thought that the cementum thickens continuously throughout a person's life.^[17]

Between the ages of 16 and 70, the thickness of cementum increases threefold. The incremental lines in the acellular cementum are thin, even and closely organized. The cellular cementum forms faster, and the incremental lines are thicker, irregular and farther apart. The appearance of incremental lines is caused by changes in the degree of mineralization.^[18]

The first use of cementum in human age estimation began with measurements of the width of the total cementum layer, rather than the number of incremental lines.^[19] In contrast to our study, Condon *et al.*,^[20] and Renz *et al.*,^[21] in their studies indicated that premolars are a more reliable age indicator. Whereas Lipsinic *et al.*, suggested using maxillary bicuspid have the best correlation for annulations count.^[22]

In this study, we counted cementum annulations in the root's middle third region. The cementum in the mid root region of a tooth is usually acellular, undisturbed and even in growth, allowing annulations to be counted easily and without difficulty. This is in contrast to Huffman and Antoine's study, which stated that the apical region of the root was considered to be the best area to count the cementum layers because rapidly growing cellular cementum was found at the root apex, whereas slower and thinner acellular cementum layers were found in the middle and cervical regions.^[23]

In our study, phase-contrast microscopy is better among all microscopes to assess the cemental annulations. This is similar to the study conducted by Kaur *et al.*,^[24] who explained that cementum annulations were more clearly under the phase-contrast microscope as compared to the light microscope and polarizing microscope.

In the present study lower correlation in the older age group was also found both in longitudinal and transverse sections of teeth which is similar to the study done by Lipsinic *et al.*,^[22] who found decreased apposition of

cementum in individuals older than 30 years. Hence, the counting of cemental annulations for age estimation over-assessed the calculated age in the younger age group, and longitudinal sections gave a better correlation than cross-sections. Similar results were obtained by Miller and Ritz Timme the regression analyses for the specimens in the <45-year-age group had a higher correlation coefficient and the estimated ages were clustered closer to the chronologic ages.^[25,26]

In the present study, we estimated the age using light, polarized and phase-contrast microscopy, which showed high significance with phase-contrast microscopic values in both under cross-sections and longitudinal sections. This is similar to the study done by Siddharth Pundir.^[14] Whereas studies by Aggarwal *et al.*,^[11] and Joshi *et al.*,^[27] the polarized microscope was preferred over the light microscope as it showed better discernibility of the annulations.

When cemental annulations were further studied under different imbibing media like quinoline and distilled water, they found that the visibility of cemental annulations was enhanced under the quinoline.^[24] However, we did not use any imbibing media to study tooth sections under the polarized microscope in our study. Several studies have reported a straight-line relationship between age and cementum thickness. A study conducted by Miller CS *et al.*^[26] and Cipriano^[28] concluded that the data analyzed by simple regression indicated that determining chronologic age in humans from cemental annulations is not possible.

In a study done by Jankauskas *et al.*^[10] and Zander and Hurzeler,^[29] it was reported that the positive correlation was found for the combined method, all correlations had a similar standard error and that the incremental lines rather have a similar use as other methods. This view is also supported by a study by Rai and Anand^[30] and Sousa *et al.*,^[31] who suggested that instead of restricting to one particular age determination method, different techniques should be applied to establish maximum reproducibility and to provide age estimation as reliable as possible. In a study by Pinch *et al.*^[32] and Star and *et al.*,^[33] it was reported

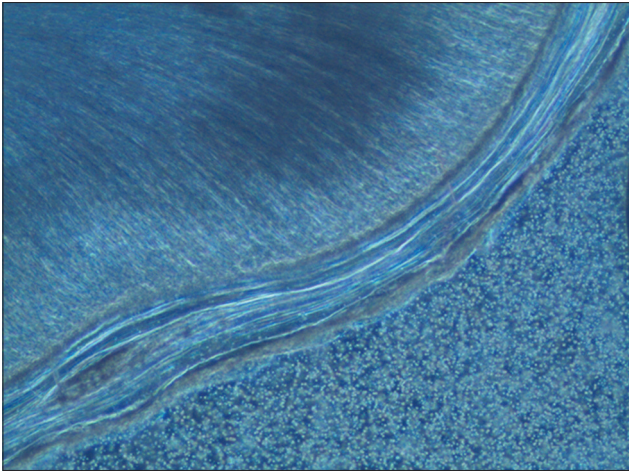


Figure 4: Ground section of teeth showing cemental lines under a phase-contrast microscope

that quantitation of cementum annuli alone is a moderately reliable means for age estimation in humans, similar to our opinion about using cemental annulations for age estimation.

However, there were limitations of the present study, which necessitates further studies considering these limitations. The method of counting cementum annulations is subjective and subject to interobserver error; clinical data for determining actual age were collected from patients, which may be unreliable; The age of tooth eruption may vary genetically from individual to individual; the field of focus for a specimen may vary when different microscopes are used, which may cause errors.

CONCLUSION

In our study, we estimated the age using various microscopes like the light microscope, a polarized microscope and a phase-contrast microscope. A positive correlation was seen between the number of cementum annulations and the actual age of an individual. Annulations counted from an image analyzer provide a close estimate of the actual age of the individual. The use of this method of counting cemental lines improves the accuracy of age estimation. Therefore, TCA age estimation by counting the lines of cementum added to the tooth's mean eruption age can be a reliable method for forensic identification and is extremely valuable in the fields of Forensic medicine, Forensic dentistry and Anthropology.

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Conflicts of interest

There are no conflicts of interest.

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