ORIGINAL ARTICLE

Estimation of age based on tooth cementum annulations using three different microscopic methods

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Abstract

Background: The hard tissues of human dentition are able to resist decay and degradation long after other tissues are lost. This resistance to decay has made teeth useful for calculation of age at death of an individual. Recent research indicates that tooth cementum annulations (TCA) may be used more reliably than other morphological or histological traits of the adult skeleton for estimation of age. **Objectives:** The purpose of this study was to examine the correlation between age and the number of incremental lines in human dental cementum and to ascertain which, among three different forms of microscopy (light, polarized, phase-contrast) was the most reliable method of studying cementum. **Materials and Methods:** The study sample consisted of 40 teeth that had been extracted from patients ranging in age from 20–70 years. Longitudinal ground sections of each tooth were prepared and examined under light microscopy, polarized microscopy, and phase-contrast microscopy. The images were magnified on a computer and the cemental lines were counted with the help of Image Analysis Pro 6.0 software. Only the dark lines were counted. **Results:** There was a strong positive correlation between the estimated age and calculated age when phase-contrast microscopy was used; the correlation was less for light and polarized microscopy. Our results suggest that there is no significant influence of sex, age, periodontal disease, or tooth type on age estimation by the TCA method. This suggests that the accuracy and repeatability of the method is not dependent on tooth type or location and that this method can be applied to the general population regardless of systemic or periodontal health. **Conclusion:** With this study we conclude that among the methods of counting incremental lines by various types of microscopy phase-contrast microscopy improves the accuracy of age estimation and may serve as a valuable aid in forensic identification.

Key words: Age estimation, cementum annulation, microscopy

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Introduction

An accurate method of age estimation is important for forensic investigators dealing with unknown bodies, parts of bodies, or skeletons. The best method for estimating the age at death from human skeletal tissue is currently unknown. The hard tissues of the human dentition are able to resist decay and degradation long after other tissues are lost. Because of this, teeth can be a useful indicator of some past variation in diet or of metabolic diseases and can also be of use for calculation of age at time of death.[1]

Cementum is the calcified tissue that surrounds the dentine and forms the attachment site for the periodontal fibers that link the tooth to the alveolar bone.[2]

Cementum is formed as a result of a continuous process throughout life and it has been shown to triple in thickness between the ages of 20 and 60 years. The thickness varies, with the maximum at the apex and the minimum near the cementoenamel junction. While acellular in the coronal portion of the root, the cementum in the apical half is both acellular and cellular.[3]

The continuous apposition of cementum may be influenced by function, occlusion, and pathologic processes. Further, functional teeth are furnished with less cementum than non-occluding teeth, while impacted teeth seem to have less cementum than functional teeth.[3]

It was hypothesized that since cemental annulations have been observed in all mammalian genera studied, they may also be found in humans and may be used to determine age in that genus. The ability to accurately estimate the ages of victims of natural or manmade disasters would be a valuable tool in forensic dentistry.[4]

The cementum consists primarily of uncalcified dense bundles of collagen fibrils. These bundles later become mineralized by hydroxyapatite crystals, whose varying orientations may be responsible for the optical effect of alternating dark and light layers.[2]

The biological explanation for the alternating layers was given by Lieberman[1] and Schroeder HE, Orale Strukturbiologie. Stuttgart- New York: Thieme; 2000[5] who suggested that the dark lines are the stop phases of mineralization during the continuing growth of fibroblasts, leading to change in mineral crystal orientation. This pattern is visible under the microscope as a series of alternating light and dark lines or bands, which are known as incremental lines of cementum.

The purpose of this study was to examine the correlation between age and number of incremental lines in human dental cementum and to ascertain whether light microscopy, polarized microscopy, or phase-contrast microscopy was the most reliable method for studying cementum.

Materials and Methods

Preparation of sections

This study was carried out in the Department of Oral Pathology, Microbiology and Forensic Odontology, Subharti Dental College, Meerut. The study sample consisted of 40 teeth; the details of which are presented in Table 1.

The teeth were collected and stored in 10% buffered formalin. Care was taken to ensure the integrity of root cementum after extraction of the teeth. The age of individuals (at extraction of the tooth) ranged from 21–70 years. Teeth were extracted because of periodontal disease, caries, or orthodontic and prosthetic reasons. Teeth with periapical pathologies were not included in the study. Signed consent from the concerned individual was taken for each tooth used in this study.

The ground sections of the teeth were prepared based on the method described by Stott *et al.* (1982).[4] The teeth were first cleaned with pumice slurry and a polishing brush in a slowly rotating handpiece. The teeth were then thoroughly washed under running water.

Table 1: Teeth included in the study group

Teeth	No.
Maxillary central incisor	2
Maxillary canine	4
Maxillary 1st premolar	10
Maxillary 2nd premolar	5
Mandibular central incisor	2
Mandibular canine	3
Mandibular 1st premolar	6
Mandibular 2nd premolar	8

Each tooth was cut into sections using a diamond-tipped disc; sectioning was done almost perpendicular to the long axis of the tooth. The sections were again rinsed under running water to clear them of debris and particles. The teeth were then ground on Arkansas stone with water to 80 μm thickness. The sections were then dehydrated, cleared with xylene, and mounted on glass slides.

Microscopy and line counting

Longitudinal ground sections of each tooth was prepared and examined under light microscope, polarized microscope, and phase-contrast microscope. In each section, the area at the junction of apical and middle third of root and the area where lines were easiest to count, irrespective of whether the cementum was cellular or acellular, was selected for counting.

Digital images of the incremental lines were taken from every section with a binocular Olympus microscope (CX-31) in bright field mode, polarizer mode, and phase-contrast mode. Micrographs were taken with a 10X objective with the help of Olympus Camedia C-5060 digital camera [Figures 1-4]. The images were magnified on the computer and the cemental lines were counted with the help of Image Analysis Pro 6.0 software.

According to other authors, a pair of light and dark lines represents 1 year.[6] In the present study, we counted only the dark lines; the line count mentioned in this study, therefore, always refers to the number of dark lines. The number of incremental lines were counted in bright field microscope, polarized microscope, and phase-contrast microscope. The lines were counted three times, separately, by the three different observers.

The eruption age of the tooth was added to the counted lines and the estimated age was obtained, as follows:

Estimated age = No. of incremental lines (n) + Eruption age of tooth (t)

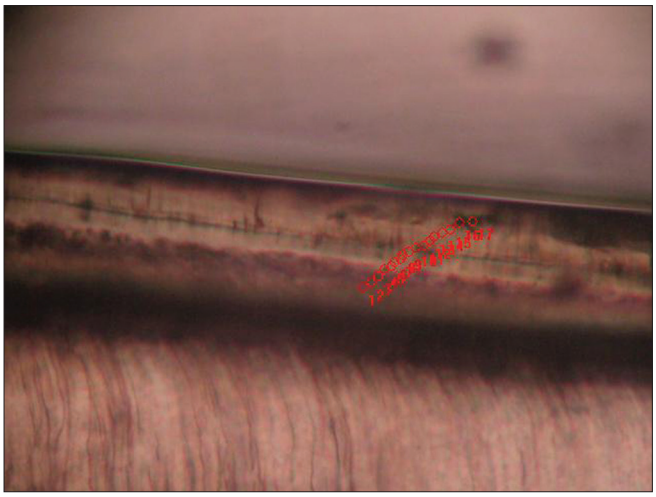

Figure 1: Photomicrograph of cemental lines by Bright field microscopy

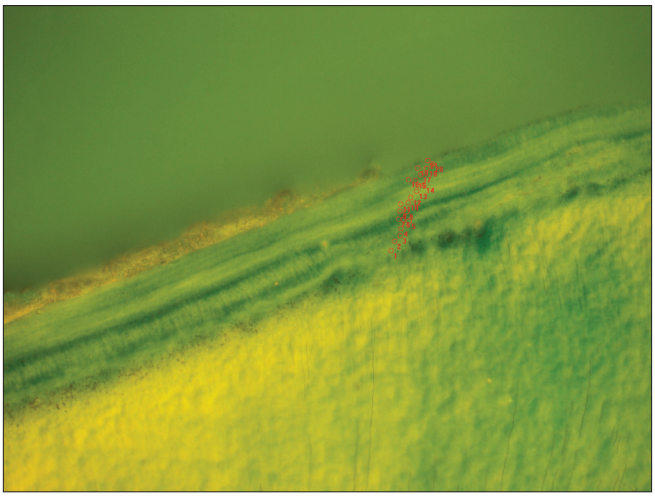

Figure 2: Photomicrograph of cemental lines by polarized microscopy

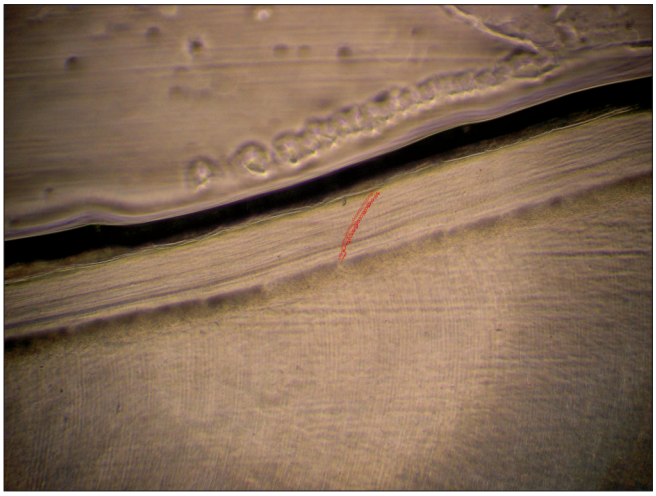

Figure 3: Photomicrograph of cemental lines by phase-contrast microscopy

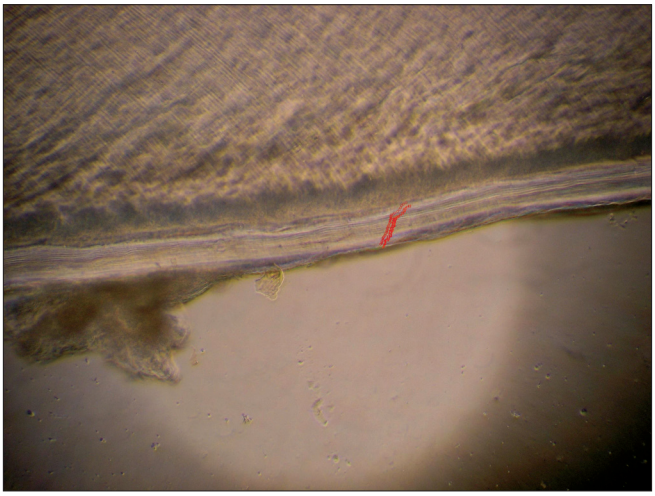

Figure 4: Photomicrograph of cemental lines by phase-contrast microscopy

The data obtained were analyzed using Student's t-test and Karl-Pearson correlation coefficient to analyse the correlation between estimated age and actual age.

Results

During sectioning or grinding, some of the sections broke completely or parts of cementum broke away; such cases were rejected. Only intact tooth sections were included in the sample. About 52 teeth were initially taken for the study but out of this 12 were rejected. Only 40 sections of 40 teeth (one section per tooth) were included in the final sample.

The actual and estimated ages of the persons based on all the three methods—light microscopy, polarized microscopy, and phase-contrast microscopy—are shown in Table 2. The Karl-Pearson correlation coefficient showed a fairly strong positive correlation between the estimated age and calculated age when phase-contrast microscopy is used; while the correlation was less with light and polarized microscopy [Table 3].

Discussion

Previous studies have evaluated the feasibility of using cemental annulations in human root cementum for age determination. Charles *et al*.[7] systematically evaluated the distribution of lines in cementum of the middle third of root in different sections. Kagerer and Gruppe[8] applied a method called 'sequential analysis,' where they tried to find the 'most stable figure' or the 'most reliable number of layers.' Wittwer-Backofen *et al*.[2] used 70–80 μm-thick unstained mineralized sections (storage time was not reported) for counting the lines, while Kagerer and Grupe[8] counted lines in cementum of 70 μm-thick unstained mineralized sections using phase-contrast microscopy.

Table 2: Cemental annulations, estimated age and actual age of each tooth sample

Specimen no.	Tooth no.	No. of annulations			Cross product			Eruption age (years)	Estimated age (years)			Actual age (years)
		LM (1)	Pol (2)	Phase (3)	1 × 2	2 × 3	1 × 3		LM	Pol	Phase	
1	23	34	38	43	1292	1634	1462	11	45	49	54	56
2	45	25	30	32	750	960	800	11	36	41	43	40
3	14	21	29	31	609	899	651	9	30	38	40	43
4	15	39	44	47	1716	2068	1833	10	49	54	57	59
5	24	17	21	23	357	483	391	10	27	31	33	36
6	24	39	42	44	1638	1848	1716	9	48	51	53	56
7	34	37	40	44	1480	1760	1628	10	47	50	54	67
8	24	28	29	31	812	899	868	9	37	38	40	43
9	24	20	28	31	560	868	620	10	30	38	41	42
10	25	29	37	39	1073	1443	1131	10	39	47	49	65
11	24	10	11	16	110	176	160	10	20	21	26	28
12	33	31	33	39	1023	1287	1209	11	42	44	50	52
13	35	29	37	40	1073	1480	1160	10	39	47	50	52
14	33	33	41	45	1353	1845	1485	11	44	52	56	60
15	44	41	45	49	1845	2205	2009	9	50	54	58	60
16	45	12	15	19	180	285	228	11	23	26	30	29
17	45	32	40	41	1280	1680	1344	10	42	50	51	54
18	44	34	40	42	1360	1680	1428	9	43	49	51	55
19	31	42	45	49	1890	2205	2058	7	49	52	56	57
20	15	20	25	29	500	750	600	10	30	35	39	42
21	11	45	48	56	2160	2688	2520	7	52	55	63	65
22	21	39	43	52	1677	2236	2028	7	46	50	59	61
23	13	28	36	41	1008	1476	1148	11	39	47	52	54
24	13	29	34	38	986	1292	1102	11	40	45	49	51
25	23	43	46	51	1978	2346	2193	11	54	57	62	63
26	15	6	7	12	42	84	72	10	16	17	20	22
27	25	10	10	11	100	110	110	10	20	20	21	22
28	33	43	46	49	1978	2254	2107	11	54	57	60	61
29	24	20	24	26	480	624	520	9	29	33	35	37
30	24	28	30	31	840	930	868	9	37	39	40	42
31	24	21	26	28	546	728	588	9	30	35	37	38
32	14	7	8	11	56	88	77	9	16	17	20	22
33	34	10	12	14	120	168	140	10	20	22	24	25
34	44	11	12	14	132	168	154	9	20	21	23	24
35	44	31	36	38	1116	1368	1178	9	40	45	47	48
36	41	44	53	55	2332	2915	2420	7	51	60	62	64
37	35	43	49	55	2107	2695	2365	10	53	59	65	67
38	35	9	11	14	99	168	154	10	19	21	24	24
39	45	40	44	47	1760	2068	1880	11	51	55	58	60
40	45	16	19	20	304	380	320	11	27	30	31	32
Mean±SD									27.40±12.0465	31.60±13.157	34.97±13.7886	

LM: Light microscope, Pol: Polarised microscope, Phase: Phase contrast microscope

Table 3: Karl-Pearson correlation coefficient between different forms of microscopy

Correlation between different forms of microscopy	Value (correlation coefficient)	*t*-value	t (38.05) standard value	*P-value*
r (Light × polarizing)	0.4734	3.72	2.030	<0.0007*
r (Polarizing × phase)	0.3539	2.6215	2.030	<0.0002*
r (Light × phase-contrast)	0.3628	2.6973	2.030	<0.0005*

*$P<0.05$ indicates a statistically significant difference

In the present study, we found that the cemental annulations were more clearly visible under the phase-contrast microscope as compared to polarizing microscopy and light microscopy. Along the axis of tooth root there are two zones of different cementum types: the acellular cementum, which mainly covers the cervical part of the root, and the cellular cementum, which mainly covers the apical part of tooth root. In the present study, we focused on the acellular cementum, which is predominantly seen in the middle third of the root.

Variations in cementogenesis, which is responsible for changing the appearance of cemental annulations, may be induced by different factors, including biomechanical forces, nutritional status, hormonal fluctuations, or ecological conditions such as temperature, ultraviolet light, humidity, altitude, or pollution.

The appearance of cemental annulations, which has been observed in more than 50 different mammalian species all over the world, has been said to reflect the natural metabolic rhythm of seasonal changes.[9-11] The seasonal rhythm in cementum annulations, as observed in alternating dark and light bands, can be explained by the metabolism of the parathyroid hormone which, in connection with vitamin D, regulates the resorption of calcium.

Thus both hormones and vitamins may interact to produce a circannual rhythm by a complex mechanism of environmental and physiochemical synchronizers.[12] Many questions remain regarding the mechanisms of tooth cementum formation and the factors influencing it; most of these questions concern the interpretation of seasonal increments.

Kvaal *et al*.[13-14] have said that apposition of cementum occurs in phases, resulting in two types of layers with different optical properties. In their study, cementum was investigated using conventional light microscopy, polarized microscopy, and phase-contrast microscopy.

The distance from one cemental line to the next represents a yearly incremental deposition of cementum in many mammals, and counting of these lines has been used routinely for estimation of age of animals. Incremental lines in cementum have also been observed in sections of human teeth and there are a number of methods for preparing and staining them for counting.

Longitudinal and transverse sections, cut from formalin-fixed human dental roots (either paraffin-embedded or frozen sections), can be stained using several techniques. The other methods include fluorescence, confocal laser scanning, interference contrast, and scanning electron microscopy.[13]

Incremental lines in cementum can be observed in decalcified sections by using toluidine blue, cresyl violet, hematoxylin, or periodic acid-Schiff stains by conventional light microscopy, but the results are not satisfactory. Since incremental lines are not destroyed by acids and stain differently than the remaining cementum, it is likely that they possess an organic structure which differs from that of the cementum.[13-14]

Klevezal and Kleinenberg[15] found that the number of incremental lines counted on a sectioned tooth equalled the age in years. The robustness of the incremental lines varies among species; in some, the lines may be difficult to count, especially in smaller species.

Phillips *et al*.[16] examined incremental lines in two species of bats (*Myotis lucifugus* and *M velifer*) whose age was know. They found that the number of incremental lines observed depended on the tooth that was extracted and on the sections examined, and suggested that several factors, such as mechanical stress and dental drift, can affect the temporal patterns of appositional growth, resulting in non-annual cycles of dentin and cementum deposition.

In this study, we confirmed the visibility and countability of lines in sections from the middle third of the root. In humans, cemental annulations are present and can be indicative of age. Any tooth or series of teeth can be used as long as the cementum is intact.

Conclusion

Countable cemental annulations are present in human teeth. Annulations counted from a photograph or an image analyzer provides a close estimate of the actual age of the individual from whom the tooth was extracted.

The use of this method of counting cemental lines improves the accuracy of age estimation and even makes age estimation possible in cases where only poorly preserved skeletal fragments are available. If the chronological age of the oldest individual in a historical population can be accurately determined by this method this will allow us to estimate life expectancy and the distribution of life spans within the population under study in a better way.

This study demonstrates that incremental lines are best viewed through phase-contrast microscopy, as opposed to polarized and bright field microscopy, and this method may serve as a valuable aid for forensic identification.

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