10.5005/jp-journals-10037-1090

ORIGINAL ARTICLE

Phase-contrast Microscopy: An Adjuvant Tool to assess Cementum Annulation in Forensic Dentistry

[1]Shilpa C Natesan, [2]Rekha Krishnapillai, [3]Bindhu P Ramakrishnan, [4]Priya Thomas

ABSTRACT

Introduction: Teeth are one of the most durable structures in human body. As they do not degrade easily due to their high mineral content, dental hard tissue has been a valuable aid in forensic identification.

Aim: The aim of this research was to estimate the age with incremental lines in human dental cementum using phase-contrast microscope and to correlate the estimated age with the actual age of the person.

Materials and methods: The study sample consisted of 30 teeth that were extracted from patients ranging from 30 to 66 years of age. Longitudinal ground sections of each tooth were prepared and examined under phase-contrast microscope. These areas were photographed, and the images were magnified in a computer. Counting of the cemental annulations were done using Image J software.

Results: The Karl-Pearson correlation coefficient showed a strong positive correlation between the estimated age and calculated age ($r = 0.928$, $p < 0.0005$).

Conclusion: Our results showed that the cemental annulations viewed under phase-contrast microscope can be used as a valuable aid for forensic identification.

Keywords: Age estimation, Incremental lines of cementum, Phase-contrast microscope, Tooth cementum annulations.

How to cite this article: Natesan SC, Krishnapillai R, Ramakrishnan BP, Thomas P. Phase-contrast Microscopy: An Adjuvant Tool to assess Cementum Annulation in Forensic Dentistry. Oral Maxillofac Pathol J 2017;8(1):5-8.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Tooth as an indicator of age dates back to first half of 19th century. Yet the best method for estimating the age at death from human dental tissue is unknown. Age determination can be of great value in forensic odontology for individual identification in natural calamities, crimes, or bomb blasts. All hard tissues of the tooth can be used for age estimation out of which cementum has shown to provide more accurate and reliable results than others. Zander and Hurzeler[1] stated that cementum is potentially a better age-estimating tissue due to its unique location in the alveolar process.

Cementum is a calcified tissue that surrounds the root dentin of teeth and is formed as a continuous process throughout life. In cementum, there are alternating dark and light bands. These dark lines have been referred to as incremental lines of cementum/"Lines of Salter" and the cementum between the two dark lines have been referred to as bands. These are highly mineralized areas representing the periods of rest during the process of cementogenesis. According to Lieberman, a pair of light and dark lines represents one year.[2] Variations in lines may be induced by biomechanical forces, nutrition, hormonal cycle, or ecological conditions, such as temperature, Ultraviolet (UV) light, humidity, altitude, or pollution.[3]

Cemental annulations can be a better tool for dental age estimation for forensic purpose due to the following reasons: (1) Hard tissues of human dentition are able to resist decay and degradation long after other tissues are lost; (2) location of cementum within the alveolar process; (3) the incremental lines are more clearly visible in cementum than that of enamel or dentin; (4) cementum is more resistant to resorption than bone; (5) any tooth or series of teeth can be used as long as the cementum is intact.

MATERIALS AND METHODS

Collection of Specimens

The study was carried out in the Department of Oral Pathology and Microbiology, Annoor Dental College and Hospital, Muvattupuzha, Kerala. The study sample consisted of 30 extracted teeth of known age, which included representatives of all four groups: Incisors, canines, premolars, and molars. A signed consent was obtained from each individual from whom the tooth was extracted. Teeth extracted due to dental caries, orthodontic, and prosthetic reasons were included in the study while teeth with periapical pathology, root caries, developmental anomaly, hypercementosis, or root resorption were excluded. An ethical committee clearance was obtained from our institutional review board to undergo the study.

[1]Postgraduate Student, [2,3]Professor, [4]Reader

[1-4]Department of Oral and Maxillofacial Pathology, Annoor Dental College and Hospital, Muvattupuzha, Kerala, India

Corresponding Author: Shilpa C Natesan, Postgraduate Student, Department of Oral and Maxillofacial Pathology, Annoor Dental College and Hospital, Muvattupuzha, Kerala, India Phone: +918086178065, e-mail: sshilpacn@gmail.com

Preparation of Sections and Line Counting

Longitudinal ground sections of each tooth were prepared manually and examined under a phase-contrast microscope. Areas with prominent incremental lines in the mid-root region were selected for counting.

These areas were photographed, images later transmitted to a computer monitor, and counting was done with the help of ImageJ software (Wayne Rasband, National Institute of Health USA).[4]

Measurements and Calculations

First, the width of the cementum from the dentin-cemental junction to the surface of the cementum was measured in an area where the lines seemed to run approximately parallel. Then the width of two adjacent incremental lines which were most accentuated was measured, and the number of incremental lines in the total cementum width was calculated (Figs 1 and 2).

$$\text{Number of incremental lines (n)} = \frac{X}{Y}$$

where X is the total width of cementum (from dentinoenamel junction to cementum surface) and Y is the width of cementum between the two incremental lines. By adding average age of eruption in years for each tooth as presented in Wheeler's Dental Anatomy with the counted number of incremental lines, the chronological age of the individual was obtained.[5,6]

$$E = n + t$$

where estimated age (E) = number of incremental lines (n) + eruption age of tooth (t).

RESULTS

A total of 35 extracted teeth were included in the study out of which only 30 had clear, visible and distinct cemental lines. The data obtained for these 30 specimens were tabulated (Table 1) and statistical analysis was carried out. The Karl Pearson's correlation coefficient showed a strong positive correlation between the estimated age and the actual age of the tooth specimens ($r = 0.928$, $p < 0.0005$).

DISCUSSION

Layering in cementum has been identified in virtually every group of mammals and their use in age determination is routine among wildlife biologists.[6] Stott et al[7] in 1982 first used tooth cementum annulations as an age estimation method in humans. Later Grosskopf[8] in 1989 showed that the method was also applicable to historical skeletons and cremations. Since then a lot of studies have been carried out to find a positive correlation between age of the person and incremental lines of cementum. Though several have succeeded in this aspect, there are negative results which question the reproducibility of this method.

Cementum is deposited in an irregular rhythm resulting in unevenly spaced incremental lines. In acellular cementum these lines tend to be close together, thin, and even. In the more rapidly formed cellular cementum, the lines are further apart, thicker, and more irregular. The appearance of incremental lines in cementum is mainly due to differences in the degree of mineralization and recent evidence suggests that they are rich in osteopontin.[9] These lines are assumed to be added yearly.

There had been a debate among researches over the apt area to count the incremental lines in the past. Some considered the middle third to yield more accurate and reproducible results while others chose the apical area. In the present study mid-root section was chosen for the following reasons:

- The thickness, width, and cellularity of the layers of cementum increases apically, thereby complicating the counting of annulations.

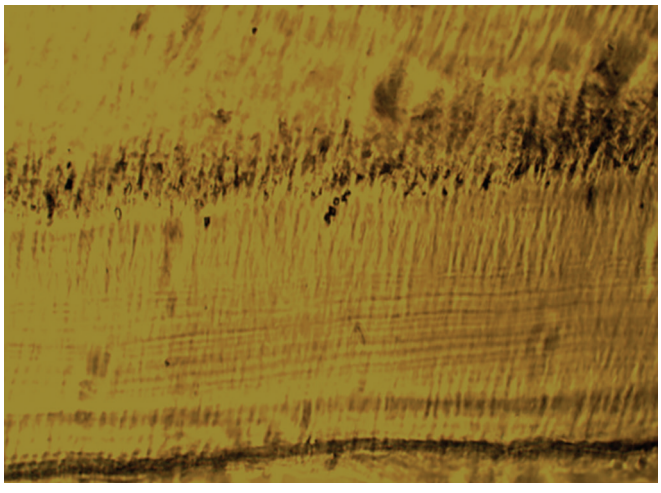

Fig. 1: Photomicrograph showing incremental lines of cementum in longitudinal ground section under phase-contrast microscope

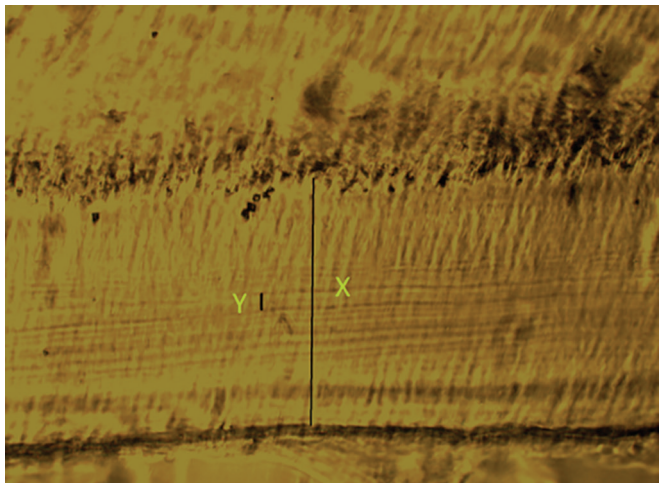

Fig. 2: Photomicrograph showing measurements of total cementum width (X) and one incremental bandwidth (Y) using image analysis software

Table 1: Counts of cemental annulations with eruption time estimates compared with actual age of individuals (in years)

Specimen number	*Tooth number*	*Thickness of cementum (mm)*	*No. of annulations*	*Eruption range (years)*	*Estimated age (years)*	*Actual age (years)*	*Mean age error (years)*
1	14	8897.55	34	10–11	46	45	1
2	22	1961.96	47	8–9	56	57	1
3	31	776.00	49	6–7	56	60	4
4	43	754.38	54	9–10	60	61	1
5	16	838.53	47	6	54	50	4
6	12	600.66	33	8–9	40	45	5
7	11	756.89	48	7–8	56	56	0
8	13	1752.35	46	11–12	58	57	1
9	12	486.13	46	8–9	55	61	6
10	22	708.51	49	8–9	57	62	5
11	32	571.20	44	7–8	52	62	10
12	11	599.15	57	7–8	65	62	3
13	23	1021.57	51	11–12	62	62	0
14	21	465.14	43	7–8	51	51	0
15	42	416.00	42	7–8	49	47	2
16	13	434.19	30	11–12	41	45	4
17	22	573.82	44	8–9	53	52	1
18	41	644.26	35	6–7	42	47	5
19	33	658.74	38	9–10	47	49	2
20	22	828.53	43	8–9	53	49	4
21	21	1013.13	46	7–8	53	57	4
22	11	892.50	58	7–8	66	60	6
23	21	1137.62	44	7–8	51	52	1
24	33	721.54	43	9–10	53	56	3
25	26	954.23	54	6	60	66	6
26	23	865.53	50	11–12	62	59	3
27	32	475.11	24	7–8	32	33	1
28	31	424.56	23	6–7	30	30	0
29	26	500.84	28	6	34	36	2
30	21	562.22	31	7–8	39	39	0

- The number of resorption areas also increases apically.
- The thinness of the cementum near the neck of the tooth inhibits scoring.
- To minimize the influence of factors known to obscure annulations or produce variation in cementum, such as hypercementosis due to local or systemic disease.

The middle-third, therefore, represents the best compromise as far as layer width, cellularity, and resorption are concerned.[10]

In the present study, phase-contrast microscope was used in the light of the experiment conducted by Pundir et al[11] using three different microscopes: Polarized microscope, light microscope, and phase-contrast microscope. There was a strong positive correlation between estimated age and actual age with phase-contrast microscope as compared with the other microscopes in their study. The study correlates with those conducted by Aggarwal et al[12] in that there is no significant influence of sex, periodontal disease, or tooth type on the estimation quality of the total cemental annulations method.

Out of 30 specimens selected for the study, four of them showed mean age error >5. All of these four specimens were from patients over 60 years of age. The decreased correlation as the age advances can be probably explained by the changes in cementum apposition.[13] Out of 30 specimens, 18 of them showed a mean age error ≤ 1. So, 60% of the specimens give a fairly reliable result.

An ambiguity still exists among researchers in the method of sectioning (longitudinal and cross-sectional) of the tooth for better perceptibility of cemental annulations. Each of these methods has its own advantages and limitations. Klevezal and Kleinenberg[6] have favored longitudinal sections since the entire length of the root is in view in this method. In a study conducted by Avadhani et al,[14] it was observed that transverse sections give better visibility and accountability of incremental lines. To view the entire thickness and length of the root portion of the tooth, longitudinal sections have been preferred in our study.

This technique makes age estimation possible in cases where only poorly preserved skeletal fragments

are available.[11] It also gives indication of events, such as pregnancies, skeletal trauma, and renal disorders which could be accurately dated to an individual's life history and thus aid in identification.[15] A major disadvantage of this technique is the necessity to extract and section the teeth. Moreover, specialized microscopes may be required for detailed view of the cemental lines.

CONCLUSION

This method can be applied to the general population regardless of systemic or periodontal health. Cemental annulations, when appreciated, can be used as a valuable aid for forensic identification. Though a lot of research has been done on this aspect of cementum by a number of researchers, a standard method for preparation of sections has not yet been advocated. Also, the effects of periapical pathology and developmental anomaly of teeth on incremental lines of cementum need to be evaluated. The possibilities for counting cementum annulations with fully automated software and a more accurate computer-generated formula for calculations on a large population need to be further explored.

REFERENCES

1. Zander HA, Hurzeler B. Continuous cementum apposition. J Dent Res 1958 Nov-Dec;37(6):1035-1044.
2. Lieberman DE. The biological basis for seasonal increments in dental cementum and their application to archaeological research. J Archaeol Sci 1994 Jul;21(4):525-539.
3. Wittwer-Backofen U, Gampe J, Vaupel JW. Tooth cementum annulation for age estimation: results from a large known-age validation study. Am J Phys Anthropol 2004 Feb;123(2):119-129.
4. Ash MM, Nelson SJ. Wheeler's dental anatomy, physiology, and occlusion. 8th ed. Philadelphia: Saunders; 2003.
5. Nelson, Stanley J. Wheeler's dental anatomy, physiology and occlusion. Elsevier Health Sciences; 2014.
6. Klevezal, GA.; Kleinenberg, SE. Age determination of mammals from annual layers in teeth and bones. Springfield (VA): US Department of Commerce; 1967.
7. Stott GG, Sis RF, Levy BM. Cemental annulation as an age criterion in forensic dentistry. J Dent Res 1982 Jun;61(6):814-817.
8. Grosskopf B. Incremental lines in prehistoric cremated teeth. A technical note. Z Morphol Anthropol 1989;77(3):309-311.
9. Berkovitz, BKB.; Holland, GR.; Moxham, BJ. Oral anatomy, histology and embryology. 4th ed. Oxford: Mosby Elsevier; 2009. p. 172.
10. Charles DK, Condon K, Cheverud JM, Buikstra JE. Cementum annulation and age determination in homo sapiens. I. Tooth variability and observer error. Am J Phys Anthropol 1986 Nov;71(3):311-320.
11. Pundir S, Saxena S, Aggrawal P. Estimation of age based on tooth cementum annulations using three different microscopic methods. J Forensic Dent Sci 2009 Jul;1(2):82-87.
12. Aggarwal P, Saxena S, Bansal P. Incremental lines in root cementum of human teeth: an approach to their role in age estimation using polarizing microscopy. Indian J Dent Res 2008 Oct-Dec;19(4):326-330.
13. Solheim T. Dental cementum apposition as an indicator of age. Scand J Dent Res 1990 Dec;98(6):510-519.
14. Avadhani A, Tupkari JV, Khambaty A, Sardar M. Cementum annulations and age determination. J Forensic Dent Sci 2009 Jul;1(2):73-76.
15. Kagerer P, Grupe G. Age-at-death diagnosis and determination of life-history parameters by incremental lines in human dental cementum as an identification aid. Forensic Sci Int 2001 Apr;118(1):75-82.