JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, May 2010, Vol. 55, No. 3
doi: 10.1111/j.1556-4029.2010.01363.x
Available online at: interscience.wiley.com

TECHNICAL NOTE

ODONTOLOGY

Sowmya Kasetty,[1] *B.D.S., M.D.S.; M. Rammanohar,*[2] *B.D.S., M.D.S.; and T. Raju Ragavendra,*[3] *B.D.S., M.D.S.*

Dental Cementum in Age Estimation: A Polarized Light and Stereomicroscopic Study

ABSTRACT: Dental hard tissues are good candidates for age estimation as they are less destructive and procedures to determine age can be easily performed. Although cementum annulations and cementum thickness are important parameters in this regard, they are seldom used. This study was undertaken to review the methods, difficulties in execution of techniques, and accuracy of cementum thickness and annulations in estimating the age. Unstained and stained ground sections of tooth were used to measure cemental thickness and count cemental annulations based on which age was estimated and was compared with known age. Although there was positive relation between cemental thickness and annulations with age, only in 1–1.5% of cases, age could be predicted with accuracy.

KEYWORDS: forensic science, forensic odontology, age estimation, cementum annulations, cementum thickness, polarized microscope, stereomicroscope

The estimation of age plays an important role in the forensic identification. Dentition is one of the four systems used in estimating physiologic age, the other three being bone development, secondary sexual characteristics, and stature and weight. Once the full complement of dentition is in place, the age estimation based on dentition is not possible and has to depend on the changes within the dental hard tissues (1).

Among the three dental hard tissues, cementum continues to be deposited slowly throughout life and its thickness increases by about threefold between the ages of 16–70 years (2). Therefore, one may be able to estimate the age based on apposition of cementum on the surface of dental roots. Studies have appeared in literature correlating the age with cementum thickness (3,4) and with cementum annulations (5–8). Most of these studies have found a positive correlation between these parameters and age, suggesting that these parameters can be helpful in age assessment. From forensic point of view, these age estimation methods should be very accurate to be used for medicolegal purposes.

This study was undertaken to review these methods and identify the difficulties with these techniques, and accuracy of cementum thickness and cemental annulations in estimating known age is evaluated. Polarized light microscope was preferred over light microscope as it showed better discernability of the annulations. Usefulness of stereomicroscope and image analysis software for accurate measurement of cementum thickness is evaluated.

[1]Department of Oral Pathology and Microbiology, People's College of Dental Sciences and Research Centre, Madhya Pradesh, India (former PG student, College of Dental Sciences, Davangere, Karnataka, India).
[2]Educare Institute of Dental Sciences, Kerala, India.
[3]Department of Oral Pathology and Microbiology, People's Dental Academy, Madhya Pradesh, India (former PG student, College of Dental Sciences, Davangere, Karnataka, India).

Received 5 Jan. 2009; and in revised form 15 April 2009; accepted 2 May 2009.

Materials and Methods

The study was conducted on 200 tooth specimens that were stored in 10% formalin. For a wide representation of age, 40 specimens each from second decade to sixth decade were selected. Molars were excluded from the study because of difficulty in sectioning of the tooth. In the case of maxillary first premolar, the average of two root width measurements was taken.

Two longitudinal sections of 100μ thick were prepared from each tooth using a hard tissue microtome (Leica SP1600, Nussloch, Germany). One unstained section was observed under polarized microscope (BX51, Olympus, Tokyo, Japan) at 10× magnification attached to the computer (Fig. 1). Cementum annulations were counted using image analysis software (Proplus version 4.1.0.0; Media Cybernetics, Bethesda, MD). From the number of annulations, age was estimated using formula, estimated age = eruption age of the tooth + number of cementum annulations in that particular tooth. The data obtained were statistically analyzed for age prediction by simple regression equation (Table 1).

The other section was stained with Alizarin red stain, and image was captured using stereomicroscope (SZX12, Olympus) at 5× objective to measure the cementum width (Figs. 2 and 3) using image analysis software. Cementum thickness was measured at four areas of the tooth (Fig. 4).

C1: CE1 + CE2 (thickness of cementum at 1/3 of root length from apex of lingual side + thickness of cementum at 1/3 of root length from apex on labial side).

C4: CE3 + CE4 (thickness of cementum apically on lingual side + thickness of cementum apically on labial side).

C1 and C4 were taken as they represent the cementum thickness together on labial and lingual side at that particular region.

The data obtained were tabulated and were used for statistical analysis of age prediction by multiple regression equation (Table 4).

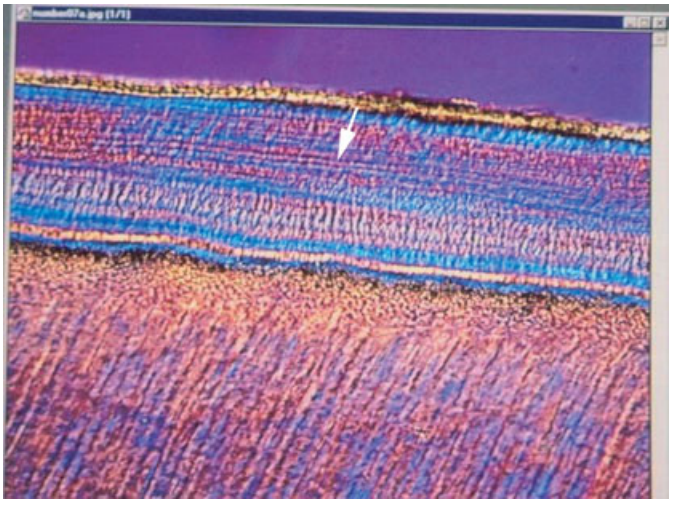

FIG. 1—*Arrow depicting cementum annulations in polarized microscope.*

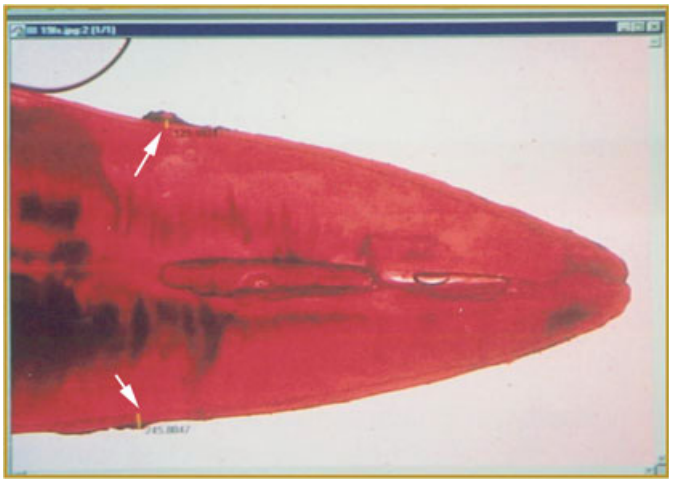

FIG. 2—*Stereomicroscopic picture showing CE1 and CE2 measurements (arrows).*

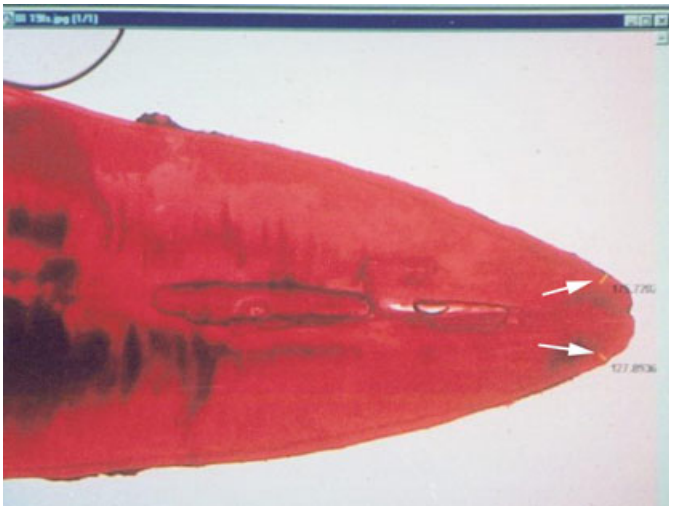

FIG. 3—*Stereomicroscopic picture showing CE3 and CE4 measurements (arrows).*

Results and Discussion

Cementum Annulations and Age

In this study, an attempt was made to find the relationship between the number of annulations and age. Correlation coefficient between estimated age and known age was calculated, and a positive correlation was observed, which was found to be statistically significant ($r = 0.42$) (Table 1) which correlates with the study of Lipsinic et al. (9) where the correlation coefficient for 42 specimen was 0.51. However, in the study by Stein (4), where 52 teeth were used, a much higher correlation between predicted age and known age was seen ($r = 0.93$).

For 200 teeth, the predicted age range was ±12 years, and only in 1.5% of cases was there an exact correlation between the known and predicted age. This makes an accurate prediction difficult (Fig. 5).

In this study, an attempt was made to find the relationship between the number of annulations and age range >35 and ≤35 years and a positive correlation were found between number of annulations and age in both the age groups (Table 2). In this study, the highest known age was 60 years. This finding is in accordance with the study of Lipsinic et al. (9) and Stein (4) and in these studies the correlation was lower in persons older than 60 years.

Problems Encountered During Counting the Cementum Annulations

- Incremental lines are not always seen as distinct lines as there is incomplete separation of lines.
- There was variation in thickness of lines.
- Same lines in deeper planes may get projected as another line.
- Resorption of cemental surface reduces thickness of cementum.
- Cemento-dentinal junction is not always distinct and so the lines here are not distinct.

For this reason, 51 individual teeth were counted for annulations by two independent observers for testing the consistency in counting the annulations and reliability coefficient was found to be 0.97, the coefficient of determination r^2 was 90%, and coefficient

TABLE 1—*Relationship between cementum annulation and age.*

Particulars	Known Age	Annulations	Eruption Age	Estimated Age (Annulation + Eruption Age)	Predicted Age
Range	17–60	8–42	7–12	16–53	24–53
Mean	35.9	21.0	10.3	31.3	36.0
SD	13.5	6.7	1.8	7.0	5.6
Regression equation (prediction of age)	–	–	–	$r = 0.42^{*}$ $p < 0.001$	Age = 11.1 + 0.795 (Estimated age) predicted Range = ±12 years

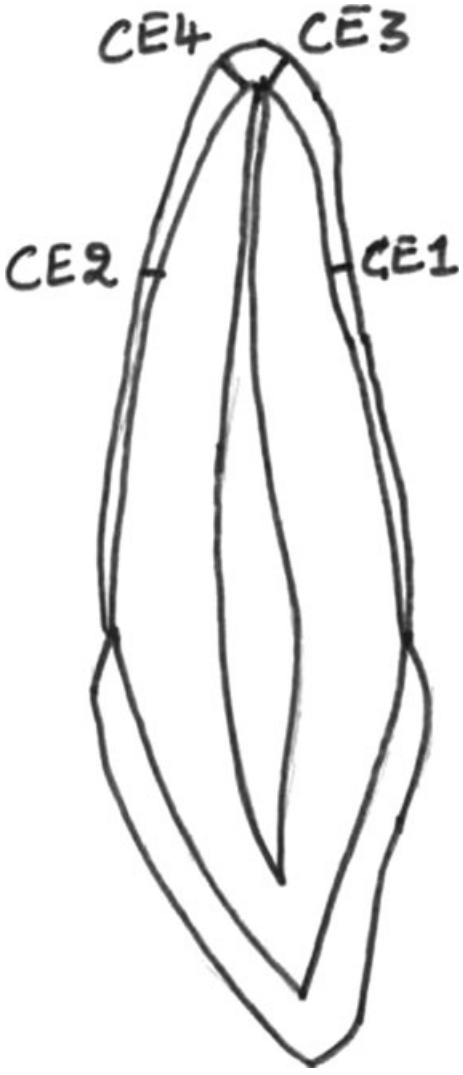

FIG. 4—*CE1: thickness of cementum in μm at 1/3 of root length from apex on lingual side. CE2: thickness of cementum in μm at 1/3 of root length from apex on labial side. CE3: thickness of cementum in μm apically on lingual side. CE4: thickness of cementum in μm apically on labial side.*

correlation was 0.95 (Table 3). This confirms that counting of annulations is relatively accurate.

Cementum Thickness and Age

In this study, correlation between cementum thickness and age was found to be statistically significant (Table 5). This is in accordance with studies by Solheim (3) and Johanson (10).

Cementum thickness in different areas of maxillary and mandibular teeth showed different correlation with age. For maxillary teeth, C1 showed the strongest correlation with age ($r = 0.46$) (Table 4). This supports the findings of Solheim (3) who observed that cementum thickness at 1/3 of the root length (C1) is a more accurate predictor of age than cemental thickness at the apex (C4). But for the mandibular teeth cementum thickness at apex (C4) showed a stronger correlation compared to cementum thickness at 1/3 of root length from apex (C1) (Table 4). This was in accordance with the study of Johanson (10) who found that apical cementum thickness was better for age estimation in mandibular teeth ($r = 0.55$).

TABLE 2—*Annulations with age range of >35 years and ≤35 years.*

		Annulations		
Age Range	No. of Cases	Range	Mean	SD
>35 years	88	9–34	18.7	5.4
≤35 years	112	8–42	22.7	7.1

t-test, significance $t = 4.38$, $p < 0.001$.
The value is significant if $p < 0.05$.

TABLE 3—*Inter-observer reliability in age assessment based on cementum annulations.*

Annulations	Observer – 1	Observer – 2
Mean ± SD	18.20 ± 6	18.16 ± 8
Range	7–35	5–39

Correlation coefficient (r) = 0.95.
Coefficient of determination (r^2) = 90%.
Reliability coefficient = 0.97.

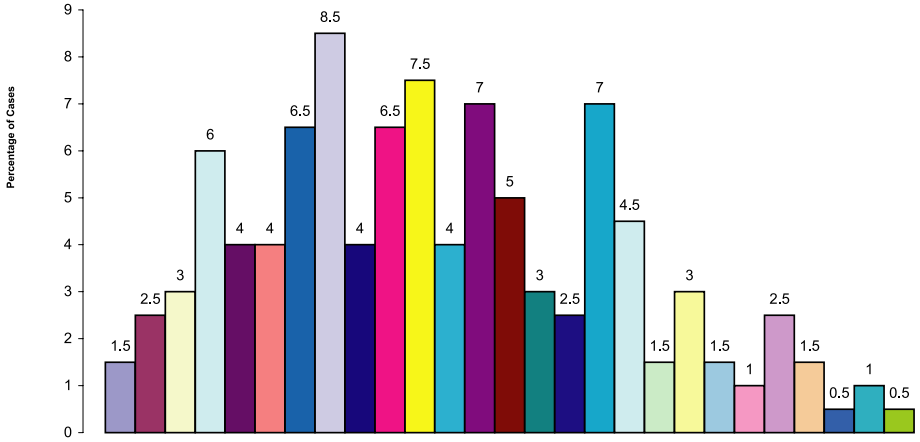

FIG. 5—*Difference between the known age and predicted age based on cementum annulations.*

TABLE 4—*Relationship of cementum thickness with age.*

		CE1	CE2	CE3	CE4	C1 (CE1 + CE2)	C4 (CE3 + CE4)	Prediction of Age (for given C1 and C4)**
Maxillary (114)	Mean ± SD	151.9 ± 95.2	160.7 ± 95.2	379.6 ± 362.3	354.2 ± 246.5	312.5 ± 154.5	733.8 ± 522.1	Age = 22.4 + 0.036 (C1) + 0.003 (C4)
	r value*	0.35	0.42	0.20	0.34	0.46	0.30	
	p value	$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.01$	
Mandibular (86)	Mean ± SD	136.0 ± 70.4	163.8 ± 95.1	344.8 ± 214.2	364.7 ± 228.6	299.7 ± 136.0	709.5 ± 415.8	Age = 25.7–0.001 (C1) + 0.016 (C4)
	r value*	0.07	0.28	0.37	0.54	0.23	0.49	
	p Value	$p = 0.53$, NS	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.001$	

*Pearson's correlation coefficient. The value is significant if $p < 0.05$ and $p < 0.01$.

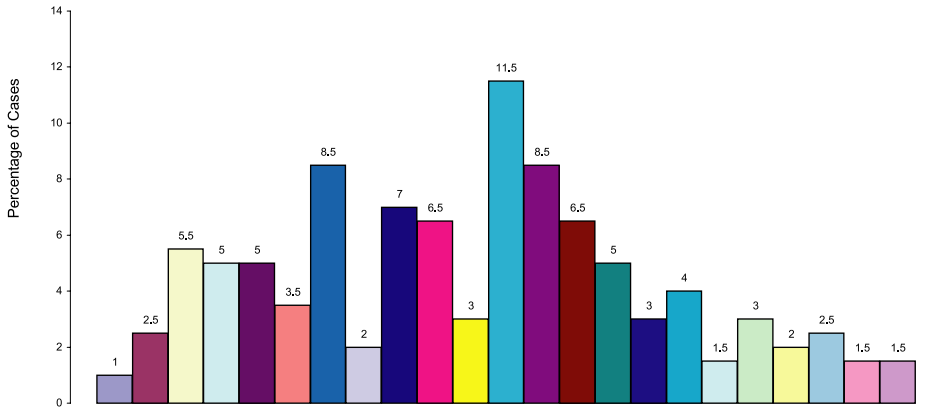

FIG. 6—*Difference between the actual age and predicted age based on cementum thickness.*

Although there was strong correlation between cementum thickness and age, only in 1% of cases was there an exact correlation between predicted age and known age (Fig. 6).

Cementum thickness with the age range >35 and ≤35 years for both maxillary and mandibular teeth was found to be statistically significant (Table 5). This is in accordance with the study by Solheim (3) and Johanson (10). However, they found a greater divergence from known age in persons older than 60 years. Analysis has also shown that women had slightly greater cementum apposition than men; however, results were statistically not significant. This finding was in contrast to Solheim (3) who stated that women showed a lesser deposition of cementum.

When the correlation coefficient of cementum annulations and age was compared with cementum thickness and age, the *r* values were found to be 0.42 and 0.76, respectively. The coefficient of determination/dependency (*r*) was 18% and 58% for cementum annulation and thickness methods, respectively. The difference between two correlation coefficients was calculated using *Z*-test, which was 2.4, and was found to be statistically significant (Table 6). Hence, in this study, the cementum thickness showed a far higher correlation with age than cementum annulations.

TABLE 6—*Significance of difference between two independent correlation coefficients of two different methods.*

Method	Relationship Between	Correlation Coefficient (*r* value)	r^2	Difference Between Correlation Coefficients*
I	Estimated age (annulations) and age	0.42 $p < 0.01$	18%	$Z = 2.4$ $p < 0.05$ sig.
II	Cementum thickness and age (C_1 and C_4)	0.76 $p < 0.001$	58%	

*Z-Test (normal curve test).

Conclusion

In this study, a large sample was used, which represented all the age groups. Very few studies are available in the literature where these two methods for age estimation have been compared.

Cementum thickness and number of annulations showed an increase with age, which were found to have statistical significance. Correlation of age with cementum thickness was statistically more

TABLE 5—*Cementum thickness with age range of >35 years and ≤35 years.*

Tooth	Age Range	No. of Teeth	C1 (CE1 + CE2)		C4 (CE3 + CE4)	
Maxillary	>35	55	90.4–978.8	191.0 ± 141.0	213.2–3410.4	419.9 ± 570.0
	≤35	59	157.4–868.5	374.6 ± 148.8	232.2–2954.5	759.3 ± 464.1
	t-test, significance		$t = 6.66$, $p < 0.001$		$t = 3.44$, $p < 0.01$	
Mandibular	>35	33	155.0–978.8	268.6 ± 160.9	213.1–2694.8	557.8 ± 451.3
	≤35	53	119.4–741.6	127.6 ± 115.3	310.7–1907.1	324.9 ± 365.5
	t-test, significance		$t = 4.76$, $p < 0.001$		$t = 2.65$, $p < 0.05$	

Significant if $p < 0.05$.

significant than with cementum annulations. However, an exact correlation between known age and predicted age with both cementum annulation method and cementum thickness method was found to be 1.5% and 1% cases, respectively.

Hence, from this study, it was found that these two methods are not sufficiently accurate for prediction of age very specifically for medicolegal and forensic purposes. The procedural difficulties like indistinct annulations and sometimes absence of annulations complicate the calculation based on these methods and make age assessment less reliable. Hence, there is need to find more accurate method of calculation for estimating age using cementum. Whether other factors like sex, race, geo-climatic condition, and nutritional status play a role in rate of cementum formation should be evaluated.

Acknowledgments

Dr. Sowmya Kasetty would like to thank his teacher Dr. R Venkatasubramanyam M.D.S., Professor & Head, Dept. of Oral Pathology, Drs Sudha & Nageshwara Rao Siddahartha Institute of Dental Sciences, Gannavaram, AP, India, for his valuable suggestions and help during this study.

References

1. Gustafson G. Forensic odontology, 1st edn. London: Staple Press, and New York: Elsevier, 1966.
2. Berkovitz BKB, Holland GR, Moxham BJ. A colour atlas and text of oral anatomy, histology and embryology. 2nd edn. London: Mosby-Wolfe, 1995.
3. Solheim T. Dental cementum apposition as an indicator of age. Scand J Dent Res 1990;98:510–9.
4. Stein TJ, Corcoran JF. Pararadicular cementum deposition as a criterion for age estimation in human beings. Oral Med Oral Surg Oral Pathol 1994;77:266–70.
5. Stott GG, Sis RF, Levy BM. Cemental annulation as an age criterion in the common Marmoset (Callithrize Jaechus). J Med Primature 1980;9(5):274–85.
6. Stott GG, Sis RF, Levy BM. Cemental annulation as an age criterion in forensic dentistry. J Dent Res 1982;61(6):814–7.
7. Rao NG, Rao NN. CCTV study of cemental annulations in determining the age from single tooth. Indian J Dent Res 1998;9(2):41–5.
8. Sousa EM, Stott GG, Alves JB. Determination of age from cemental incremental lines for forensic dentistry. Biotech Histochem 1999;74(4): 185–93.
9. Lipsinic FE, Paunovich E, Houston GD, Robison SF. Correlation of age and incremental lines in the cementum of human teeth. J Forensic Sci 1986;31(3):982–9.
10. Johanson G. Age determination from human teeth. Odontal Revy 1971;22:40–126.

Additional information and reprint requests:
Sowmya Kasetty, B.D.S., M.D.S.
Reader, Department of Oral Pathology and Microbiology
Peoples College of Dental Sciences and Research Centre
Bhanpur, Bhopal – 462010
Madhya Pradesh, India
E-mail: sowmyakasetty@rediffmail.com