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Correlation of Age and Incremental Lines in the Cementum of Human Teeth

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ABSTRACT: It has been long recognized that cementum thickness increases with age. Much literature has recently been devoted to utilizing incremental lines in cementum as an aging criterion in animals, but only one study has been done suggesting this technique in humans. Thirty-one teeth of known age were histologically stained and sectioned to observe incremental lines. Direct predictions of age based on these lines underestimated the age of older specimens. However, there was a correlation between number of lines and age, and with a large enough specimen size, a computer-generated formula for age prediction may be possible.

KEYWORDS: odontology, human identification, dentition, age estimation, human, cementum, incremental lines

Dentistry has established itself as a credible partner in the forensic sciences. Many postmortem identifications are made solely on dental evidence, and recent literature has concentrated on protocols correlating characteristics of individual teeth with age.

Since Broomell [*1*] in 1898, many investigators [*2-7*] have recognized that thickness of cementum continually increases with age, independent of functional stresses. However, Gustafson [*8*] was the first to develop a statistical method for age determination based on individual teeth. He used six changes seen in the aging dentition, one of which was apposition of cementum on the root.

Despite the insistence of some investigators [*9-12*] that cementum thickness may be affected by functional or systemic demands, Gustafson's method for age determination retains credibility in current reviews of forensic dentistry [*13*] and has been expanded upon significantly [*14-16*]. These same investigators, as well as Broomell, reported the occurrence of incremental lines in cementum. However, wildlife investigators [*17,18*] were the first to apply these lines in age determination routinely, and they have utilized this technique for many animal species [*19-31*].

Scott [*32*], in 1982, was the first to examine incremental lines as an age criterion in human teeth. He evaluated sections of nine teeth from three cadavers and reported a close correla-

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tion between actual age and a predicted age based on the number of incremental lines plus age at eruption of that tooth. In view of the potential value of this aging technique in forensic dental identification, the purpose of this investigation is to validate this aging technique using a larger sample of individuals.

Materials and Methods

Thirty-one extracted teeth from individuals of known age were collected for the study. To minimize variables, only maxillary first bicuspids were used. The criteria for collection were that the teeth did not exhibit clinical or radiographic evidence of periodontal destruction, and that the patients from whom they were obtained were not diagnosed as having any serious systemic diseases. Damage to the root surface could have resulted in an inaccurate count of incremental lines; therefore, any teeth with histologic evidence of cemental tears or clinical sulcular depth in excess of 5 mm, as measured from the cementoenamel junction, were excluded.

Collected teeth were immediately placed in a 10% formalin solution and stored until processed. At that point, specimens were designated a number/letter identifier to preclude any age association.

Some previous studies have used outwardly simple techniques for "cutting" tooth sections which could readily introduce artifacts, cemental damage, or inconsistent results. Others [*10,22,27*] have suggested that to obtain valid, consistent results, the teeth must be decalcified, microtome sectioned, and stained. A pilot project was conducted with Scott's method [*32*] and tested the effectiveness of various types of histologic stains.

The wafering technique [*32*] using undecalcified teeth and a diamond saw resulted in inconsistent sections. When stained and viewed, much artifact was apparent, as well as lines difficult to read with agreement between investigators. Next, decalcified, histologic sections were examined. Dahl's method for calcium [*33*], von Kossa's stain, silver impregnation techniques, and several vital histologic stains were tested for visualizing incremental lines in cementum by experienced staining technicians. Where specimens were inadvertently sectioned longitudinally, incremental lines could not be viewed distinctly enough for accurate counting. Only decalcified cross sections stained with double hematoxylin and eosin resulted in consistent, easily countable sections for these investigators.

Using this method, the teeth were decalcified in 10% formic acid, rinsed, dehydrated in descending grades of alcohol, embedded in paraffin, sectioned by microtome to approximately 5-μm thickness, stained in double hematoxylin and eosin, and mounted with Permount.

Sections were then examined for incremental lines under the light microscope by three investigators at $\times 100$. At least two sections were viewed for each specimen. Each section was obtained from different levels within the middle one third of the root. The number of lines recorded for that specimen by each investigator was the maximum number of lines visualized from all the histologic sections.

To evaluate the findings, the count of incremental lines for each specimen was averaged for the three investigators. This mean number of incremental lines was added to the estimated age at eruption of that tooth (10 years for females and 10.4 for males) to arrive at the predicted age for that specimen.

Results

Table 1 demonstrates the data for the study, including incremental line counts by investigator. Figure 1 is a scattergram of actual age plotted against predicted age for the study specimens, using Scott's method [*32*]. The overall correlation coefficient for this plot is 0.5098. Although the predicted plots for the younger specimens are clustered fairly closely to

TABLE 1—*Study data.*

		Lines Counted		
Specimen	Age/Sex	FL	EP	GH
1	54(M)	15	12	13
2	11(M)	3	3	2
3	11(M)	2	2	2
4	12(M)	2	2	2
5	12(M)	3	3	3
6	34(F)	34	26	28
7	11(M)	2	2	2
8	12(F)	2	2	2
9	12(F)	2	2	2
10	12(M)	3	2	2
11	12(F)	2	2	2
12	12(M)	2	2	2
13	45(M)	4	3	6
14	22(F)	4	4	4
15	22(F)	4	4	4
16	24(F)	4	4	4
18	43(M)	7	5	5
20	52(M)	8	8	8
21	37(M)	7	6	6
22	25(M)	5	5	5
25	34(F)	5	5	6
26	50(M)	6	6	4
27	26(M)	6	7	7
28	30(F)	4	4	5
29	33(M)	4	4	4
31	25(M)	7	6	6
33	11(F)	2	2	2
34	30(F)	6	6	6
40	23(M)	5	5	5
41	20(F)	3	3	4
42	60(M)	8	9	4

M = male.
F = female.
FL = Investigator 1.
EP = Investigator 2.
GH = Investigator 3.

the actual ages, as the age of the specimens increase past 30 years, this clustering tends to occur less closely. The solid line ($P = A$) indicates where the plots would be if the predicted age equalled the actual age of the specimens. It can be seen that as the age of the specimens increases, the predictions based on incremental lines in cementum lag well below the actual age. One specimen (6) in particular is widely separated from the group. Broken into age groups, the correlation for the less-than-30-year age group was 0.9294, and 0.0012 for the 30-year and greater age group.

Specimen 6, when evaluated closely, is found to vary from the y-axis mean by more than three standard deviations. To be as optimistic as possible about the potential value of incremental lines, we can evaluate the data excluding that specimen. If Specimen 6 were deleted, the correlation for the overall study would be 0.8447 with a standard error of the estimate of 8.0462 years. Since Specimen 6 is from the older-than-30 group, the correlation of the under-30-year group would be unaffected. But, the correlation of the 30-and-older group would improve from 0.0012 to 0.6361 with a standard error of the estimate of 8.5177.

Figures 2 and 3 illustrate linear regression plots of the actual ages and predictions for the

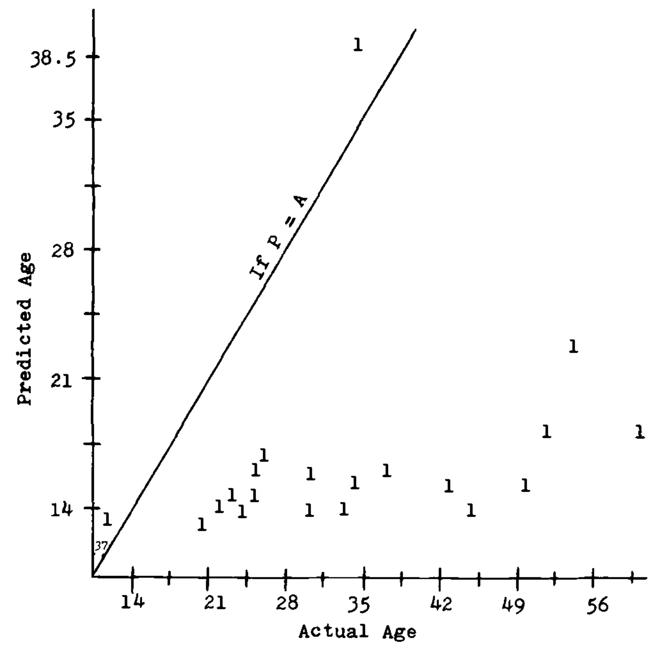

FIG. 1—*Each number denotes number of specimens at that point on the plot. The solid line denotes where the numbers would be if predicted age equalled actual ages* (P = A).

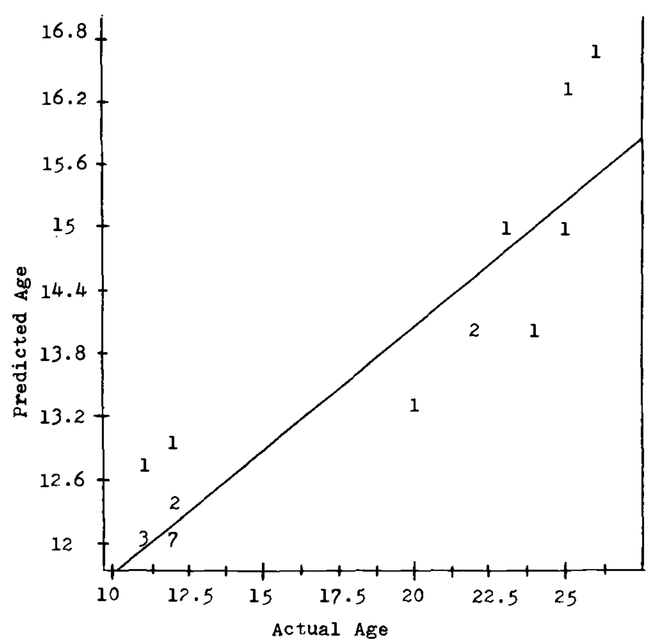

FIG. 2—*Under-30 age group, computer-generated plots of predictions. Regression line plotted. Correlation = 0.9294.*

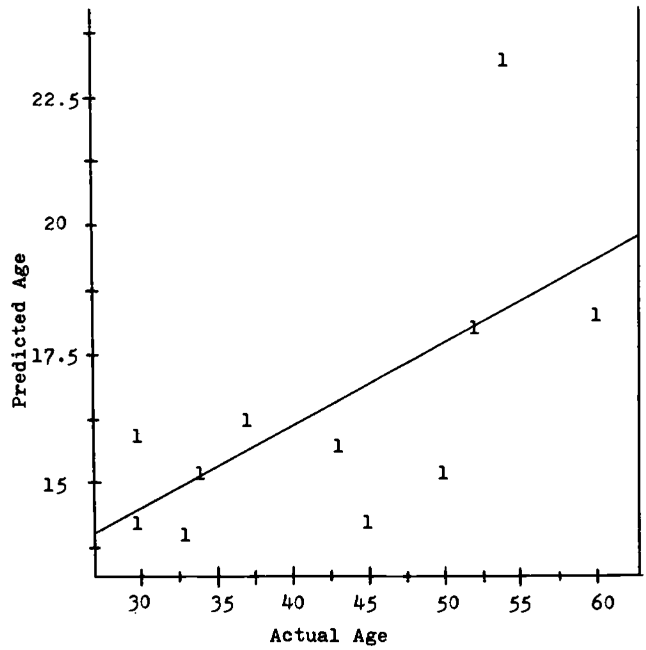

FIG. 3—*Over-30 age group, computer-generated plots of predictions. Regression line plotted. Correlation = 0.6361.*

specimens, excluding Specimen 6, based on a best-possible computer-generated formula. In the under-30 group the mean age is 16.0 and the mean predicted age is 13.1, with a standard error of the estimate of 2.2 years. However, in the 30-and-older group (Fig. 3) the mean age is 42.5, while the mean predicted age is 16.5, with a standard error of the estimate of 8.5 years.

Discussion

In many animal studies, annulations (incremental lines) have been described as paired dark and light bands occurring in the cementum [*19,20,24,26,27*]. The occurrence of these bands has been attributed to seasonal effects on nutrition, hormones, and growth status in the animal population. The dark bands are said to represent fall and winter, and the lighter bands to represent deposition during spring and summer. One must seriously question their regular occurrence in the human population, however, considering the lack of seasonal variations in nutrition and the lack of active growth past the approximate age of 25. Research is needed to explain why humans have lines in cementum at all.

Possibly, Scott's method might eventually be applied to the general population of forensic dental subjects regardless of systemic or periodontal health. Ideally, we must establish a firm scientific and statistical basis for using incremental cementum lines in a controlled situation. Therefore, the number of variables in this study was kept at a minimum by using only maxillary first bicuspids from known, systemically healthy patients with absence of periodontal destruction on the tissue to be examined.

The age at eruption figures used in this study are data from U. O. Hurme and the Forsythe Dental Center: means of 10.4 years for males, and 10.0 for females.

As illustrated by the bivariate (scatter) plot [Fig. 1], complete data for the study results in a correlation coefficient (r) of only 0.51. This correlation indicates that only 26% of the variation in the number of incremental lines was explained by the subject's age. The overall results of our data would not indicate that incremental lines are associated closely with age.

Specimen 6 is obviously widely separated from the other observations. This could be explained by undiagnosed systemic disease or increased functional demands as alluded to by Bhaskar [*10*], Glickman et al [*11*], and Gottlieb [*12*]. Figure 4 is a photomicrograph of a representative section of Specimen 6 which came from a 34-year-old female. The average incremental line count for the investigators was 29.3, which resulted in a predicted age of 39.3. This is the only specimen in the present study for the over-30 age group which even came close to predicting actual age based on the formula used by Scott. Figure 5 is a photo-

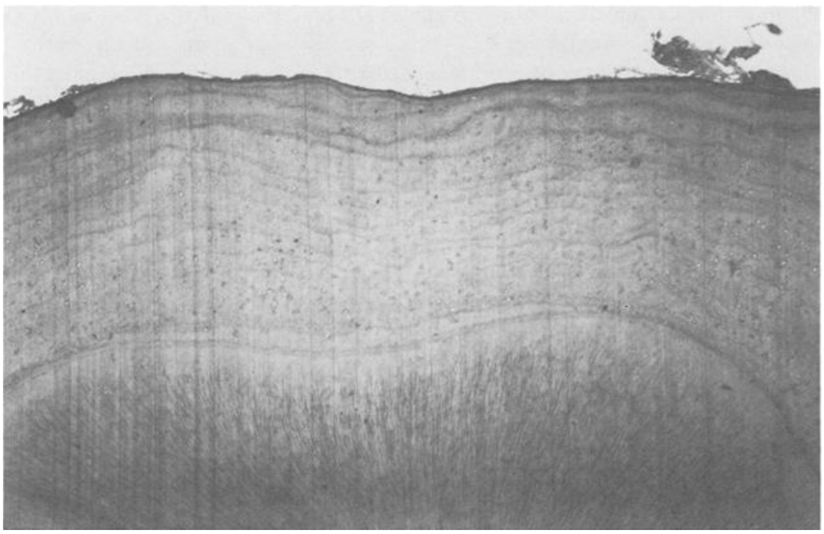

FIG. 4—*34-year-old female, ×100, Specimen 6.*

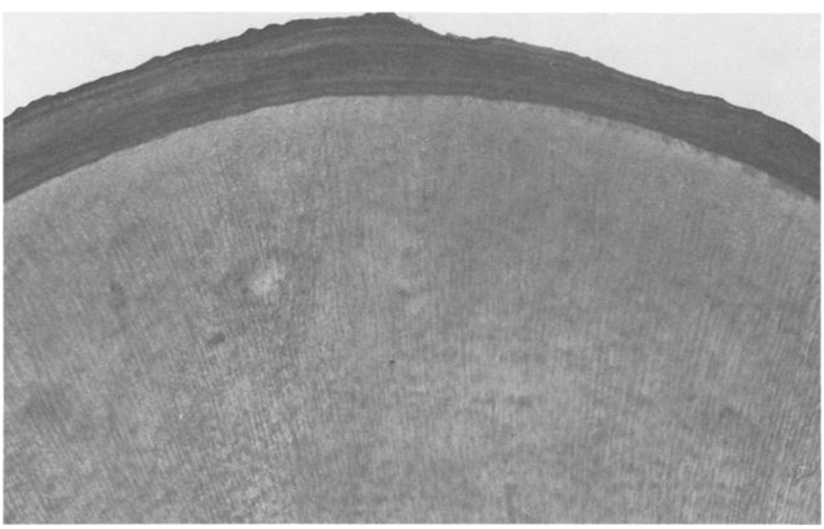

FIG. 5—*52-year-old male, ×100.*

micrograph more representative of the older age groups; a 52-year-old male where the investigators agreed on a line count of 8. This would result in a predicted age of 18.4; again, grossly underestimated.

If Specimen 6 is excluded, the overall correlation for the study becomes 0.8447, which does suggest some correlation between age and incremental lines. However, Fig. 1 reveals that even though incremental lines do increase with age, a prediction of age based directly on the number of lines plus age at eruption increasingly underestimates the older-age-group specimens. Furthermore, the correlation for this group is only 0.6361. It would appear from this study that for the over-30 age group, no formula for age prediction can be made based on incremental lines in cementum.

Even for the under-30 age group specimens in this study ($r = 0.9294$), there appears to be a problem with equal age distribution. A glance at Fig. 2 shows that a great number of the specimens are grouped around age 12. At that age, even an absence of incremental lines would result in a predicted age near the standard error of the estimate of 2.21, and therefore would heavily weigh the data toward accuracy. A lack of specimens in ages 13 to 19 is conspicuous, and predicted ages for the ages above 20 start to scatter from actual ages.

Conclusions

This study suggests that predicting age directly from maxillary first premolars by counting the number of incremental lines in cementum and adding the age at eruption of that tooth is not a reliable prediction method for humans. In this study, such a method resulted in progressively greater underprediction of the actual age as the age of the individual increased. Some type of computer-generated and adjusted formula may be possible. However, any further study should not be limited to one particular tooth, and every effort should be made to ensure that all age groups are sufficiently represented. The method using decalcified cross sections stained in hematoxylin and eosin was superior to all other staining techniques.

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References

[1] Broomell, I. N., "The Histology of Cementum," *Dental Cosmos*, Vol. 51, No. 9, Sept. 1898, pp. 697-722.

[2] Kronfeld, R., "The Biology of Cementum," *Journal of the American Dental Association* and *Dental Cosmos*, Vol. 25, Sept. 1938, pp. 1451-1461.

[3] Zander, H. A. and Hurzeler, B., "Continuous Cementum Apposition," *Journal of Dental Research*, Nov.-Dec. 1958, pp. 1035-1044.

[4] Azaz, B. et al., "Correlation Between Age and Thickness of Cementum in Impacted Teeth," *Oral Surgery*, Vol. 38, No. 5, Nov. 1974, pp. 691-694.

[5] Grant, D. and Bernick, S., "The Periodontium of Aging Humans," *Journal of Periodontology*, Vol. 43, Nov. 1972, pp. 660-666.

[6] Miles, A. E. W., *Aging in the Teeth and Oral Tissues*, Bourne, Ed., Pitman, London, 1961, pp. 383-384.

[7] Selvig, K. A., "The Fine Structure of Human Cementum," *Acta Odontology Scandanavia*, Vol. 23, 1965, pp. 423-441.

[8] Gustafson, G., "Age Determination on Teeth," *Journal of the American Dental Association*, Vol. 41, July 1950, pp. 45-54.

[9] Bodecker, C. F., "A Consideration of Some of the Changes in the Teeth from Youth to Old Age," *Dental Cosmos*, Vol. 67, No. 6, 1925, pp. 543-549.

[10] Bhaskar, S. N., *Orban's Oral Histology and Embryology*, 8th ed., C. V. Mosby, St. Louis, MO, 1976.

[11] Glickman, I. et al., "The Effect of Increased Functional Forces Upon the Periodontium of Splinted and Nonsplinted Teeth," *Journal of Periodontology*, Vol. 32, 1961, pp. 290-300.
[12] Gottlieb, B., "Histologic Consideration of the Supporting Tissues of the Teeth," *Journal of the American Dental Association*, Vol. 30, Dec. 1943, pp. 1872-1883.
[13] Cottone, J. A. and Standish, S. M., *Outline of Forensic Dentistry*, Yearbook Medical Publishers, Chicago, 1981.
[14] Johanson, G., "Age Determination from Human Teeth," *Odontolgisk Revy*, Vol. 22 (Supplement 22), 1971, pp. 55-121.
[15] Pillai, P. S. and Bhaskar, G. R., "Age Estimation from Teeth Using Gustafson's Method—A Study in India," *Forensic Science*, Vol. 3, 1974, pp. 135-141.
[16] Burns, K. R. and Maples, W. R., "Estimation of Age from Individual Adult Teeth," *Journal of Forensic Sciences*, Vol. 21, No. 2, April 1976, pp. 343-356.
[17] Scheffer, V. B., "Growth Layers on the Teeth of Pinnipedia as an Indication of Age," *Science*, Vol. 112, No. 2907, Sept. 1950, pp. 309-311.
[18] Laws, R. M., "A New Method of Age Determination for Mammals," *Nature*, Vol. 169, No. 4310, June 1952, pp. 972-973.
[19] Adams, L. and Watkins, S., "Annuli in Tooth Cementum Indicate Age in California Ground Squirrels," *Journal of Wildlife Management*, Vol. 31, No. 4, Oct. 1967, pp. 836-839.
[20] Novakówski, N. S., "Cemental Deposition as an Age Criterion in Bison," *Canadian Journal of Zoology*, Vol. 43, 1965, pp. 173-178.
[21] Crowe, D. M. and Strickland, M. D., "Dental Annulation in the American Badger," *Journal of Mammology*, Vol. 56, No. 1, Feb. 1975, pp. 269-272.
[22] Low, W. A. and Cowan, I. McT., "Age Determination in Deer by Annular Structure of Dental Cementum," *Journal of Wildlife Management*, Vol. 27, No. 3, July 1963, pp. 466-471.
[23] Keiss, R. E., "Comparison of Eruption-Wear Patterns and Cementum Annuli as Age Criteria in Elk, *Journal of Wildlife Management*, Vol. 33, No. 1, Jan. 1969, pp. 175-180.
[24] Linhart, S. B., "Age Determination and Occurrence of Incremental Growth Lines in the Dental Cementum of the Common Vampire Bat," *Journal of Mammology*, Vol. 54, No. 2, May 1973, pp. 493-496.
[25] Linhart, S. B. and Knowlton, F. F., "Determining Age of Coyotes by Tooth Cementum Layers," *Journal of Wildlife Management*, Vol. 31, No. 2, April 1967, pp. 362-365.
[26] Scott, G. G. et al., "Cementum Annulation as an Age Criterion in the Common Marmoset," *Journal of Medical Primatology*, Vol. 9, 1980, pp. 274-285.
[27] Wada, K. et al., "Determination of Age in the Japanese Monkey from Growth Layers in the Dental Cementum," *Primates*, Vol. 19, No. 4, Oct. 1978, pp. 775-784.
[28] Stoneberg, R. P. and Jonkel, C. J., "Age Determination of Black Bears by Cementum Layers," *Journal of Wildlife Management*, Vol. 30, No. 2, April 1966, pp. 411-414.
[29] Klevezal, G. A. and Kleinenberg, S. E., "Age Determination in Mammals from Annual Layers in Teeth and Bones" (translated from the Russian) 1969 Israel Program for Scientific Translations Ltd., IPST Press, Jerusalem (National Library of Medicine, Bethesda, MD), 1969, p. 128.
[30] Gasaway, W. C. et al., "Accuracy of Moose Age Determinations from Incisor Cementum Layers," *Journal of Wildlife Management*, Vol. 42, No. 3, 1978, pp. 558-563.
[31] Douglas, M. J. W., "Dental Cementum Layers as Criteria of Age for Deer in New Zealand with Emphasis on Red Deer," *New Zealand Journal of Science*, Vol. 13, Sept. 1970, pp. 352-358.
[32] Scott, G. G. et al., "Cementum Annulation as an Age Criterion in Forensic Dentistry, *Journal of Dental Research*, Vol. 61, No. 6, June 1982, pp. 814-817.
[33] Luna, L. G., "Dahl's Method for Calcium" in *Histologic Staining Methods for the Armed Forces Institute of Pathology*, McGraw-Hill, New York, 1968, pp. 175-176.

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