AGE ESTIMATION FROM DENTAL CEMENTUM INCREMENTAL LINES AND PERIODONTAL DISEASE

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ABSTRACT

Age estimation by counting incremental lines in cementum added to the average age of tooth eruption is considered an accurate method by some authors, while others reject it stating weak correlation between estimated and actual age. The aim of this study was to evaluate this technique and check the influence of periodontal disease on age estimates by analyzing both the number of cementum lines and the correlation between cementum thickness and actual age on freshly extracted teeth. Thirty one undecalcified ground cross sections of approximately 30 µm, from 25 teeth were prepared, observed, photographed and measured. Images were enhanced by software and counts were made by one observer, and the results compared with two control-observers. There was moderate correlation ((r)=0.58) for the entire sample, with mean error of 9.7 years. For teeth with periodontal pathologies, correlation was 0.03 with a mean error of 22.6 years. For teeth without periodontal pathologies, correlation was 0.74 with mean error of 1.6 years. There was correlation of 0.69 between cementum thickness and known age for the entire sample, 0.25 for teeth with periodontal problems and 0.75 for teeth without periodontal pathologies. The technique was reliable for periodontally sound teeth, but not for periodontally diseased teeth.

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INTRODUCTION

Age estimation through dental parameters can be of valuable assistance in human identification. It may also help in other situations such as: determining the legal liability of teenagers and adults of unknown age, assist adoption processes, release retirement funds for adults of unknown age as well as support research in Archeology and Paleodemography.

In individuals aged between zero and 12 years, the formation and development of the human dentition has well-defined stages which show strong correlation with chronological age. Therefore, this correlation can be used with a good degree of confidence in estimating the age of these young individuals.[1] However, this technique becomes more challenging when it is necessary to estimate the age of adult individuals with completely developed dentitions.

Dental cementum is a mineralized tissue of continuous apposition and the measurement of its thickness can help estimate the age of an individual.[2] Histological analysis of the quantity and quality of incremental lines of cementum (LC) deposited around the roots of human teeth can also help estimate the age of an individual. The LC used for estimating age are best observed in the acellular cementum, present in the cervical and middle thirds of the roots. Counting the number of alternating dark and light LC, plus the average age at which the analyzed tooth erupts provides an estimate of the chronological age of an individual. This technique was first described by Scheffer[3] and Laws,[4] who observed alternating light and dark patterns in the teeth of sea mammals: the patterns more evident in the dentine than in cementum. These patterns were found to be correlated with annual seasonal changes in feeding patterns and times of mating, allowing researchers to estimate the age of those animals with accuracy. In 1982, Stott et al.[5] applied the technique to humans, finding a positive correlation between estimated and known age.

Some authors reported failure applying the technique to humans[6-8] whilst others reported moderate correlation between age and number of LC.[9-11] Although the ultrastructural nature of LC (and the biological processes that form and change them) are not yet fully understood,[12-13] several authors report a significantly low margin of error in age estimations for this technique.[14-19]

Technical improvements[20-21] and new technologies to help differentiate the lines have been proposed,[22-23] but a decreased accuracy of the technique in more advanced ages[7, 24-25] and the influence of periodontal diseases[15-16] are still factors that require better understanding. The purposes of this study was to evaluate the correlation between the number of LC, cementum thickness and age, analyzing the influence of periodontal health on the age estimates as well as the influence of image quality and observational variation.

MATERIALS AND METHODS

Fifty-five freshly extracted teeth were obtained from 42 individuals of known age. Teeth were collected from the Dentistry College Clinic of the University of São Paulo, and from two private dental offices. The subjects answered an anamnetic questionnaire about their general health and previous known systemic conditions. All teeth were extracted for elective dental treatment. The reasons for the extractions were divided into: coronal destruction caused by caries (a), irreversible loss of periodontal attachment (b), destruction caused by caries associated with periapical processes (c), impaction (d), and orthodontic indications (e). Undecalcified cross sections of approximately 30 µm were prepared following the techniques proposed by Maat et al.[20-21] The middle third of the root was the region chosen for sectioning. Areas with evident dental calculus were avoided for sectioning. The resulting slides were mounted and photographed under an optical microscope and the resulting digital images were enhanced using *Image J* software, version 1.43s,* aiming to enhance the LC present in the image (by contrast enhancement), without alteration.[22] For measurements of cementum thickness, the scale was defined on the software, according to information provided by the manufacturer of the microscope and digital camera used for the photographs.

To analyze the possible influence of image quality on the counts, the images were classified into:[16]

1. Low quality, low contrast, many artifacts and irregular aspect of LC.
2. Moderate quality, with adequate contrast, some artifacts in the image, with regular lines.
3. Desired quality, with almost no artifacts, good contrast, regular and well defined LC.

The counting of LC was made according to Kagerer and Grupe,[16] where an observer had his scores confirmed by two other independent members (control observers) of our research group. The observer performed three counts in regions with the best visualization of the LC, and the result was considered the average between the three counts. The ages of tooth eruption[26] considered in this study were specific to the geographic region of the research (São Paulo, Brazil). The cementum thickness was measured by software in the three regions where the counts were made and in two additional different regions, and the result for cementum thickness was the average between the five measurements.

The age of the individuals and the reason for tooth extraction were not known to the observers. This data was disclosed after the age estimations and measurements were performed by the observer. The intra and interobserver variability was calculated by intraclass correlation coefficient. The correlations between known and estimated ages, and between cementum thickness and known age were calculated by Pearson's correlation coefficient. [1]

RESULTS

Of all prepared teeth, 24 teeth from 17 individuals showed no LC suitable for counting, and were discarded. The resulting sample included 31 teeth from 25 individuals with known ages between 17 and 77 years, with a mean age of 44.2 years.
The sample was divided into:-

1) Teeth extracted for any reason - but not periodontal disease, and
2) Teeth extracted because of periodontal disease.

Known and estimated ages, analyzed teeth, image grades and reasons for extraction are shown in Tables 1 and 2, respectively. Pearson´s correlation coefficient was (r) = 0.74, p<0.01, for the first group and (r) = 0.06 for the second group. For the entire sample, the correlation coefficient was (r) = 0.59.
Average thicknesses (in micrometers) for teeth extracted for periodontal reasons and non-periodontal reasons is shown in scatter plots (Graphics 1 and 2), with simple linear regression calculations for the expected values according to age.

Observational variation

For the assessment of intraobserver variation, all three observers counted five images twice with an interval of five days between counts. The intraclass correlation coefficient (c) measured the repeatability between counts. The repeatability between the counts was considered excellent[27] for the three observers. Scores for observer, control

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observer 1 and control observer 2 were 0.89, 0.98 and 0.83, respectively.

The correlation between known and estimated ages for the sample divided into images of grades 1 and 2 was 0.79 (n=17, p<0.01) and 0.59 (n=12, p<0.05), respectively. Grade 3 images were not considered because of their small number (n=2). Correlations for the sample divided into ages above or below 50 years old were 0.03 (n=13) and 0.51 (n=12, p<0.01), respectively.

The interobserver variability was also assessed (by intraclass correlation coefficient) between counts made by the observer and control observers, as seen in Table 3. The repeatability between observers' scores was considered good,[27] with a coefficient of 0.70.

DISCUSSION

During the application of the technique, observers faced many difficulties that may bias the counts, for example: variation in thickness of the LC, blurry LC on the images, overlay of the same line at different levels (that could be interpreted as two lines), lack of definition of the cementum-dentine junction and cementum resorptions (that may decrease the thickness of the cementum). In some cases, discrete changes in alternating shades of gray, highlighted by the software´s image enhancement, were the criteria used by observers to count a particular line. Despite the positive correlation found between estimated and real ages, these difficulties may have accounted for a more moderate correlation coefficient value. Even for well calibrated observers, the subjective component will always be present in the counts, even in the images with superior quality.

As repeatability for intraobserver variation was excellent, results suggest that differences between counts made from the same image, by the same observer on different occasions did not have a major influence on the errors found, as reported by other authors.[9, 17]

Despite a significant influence of subjectivity on the scores, intraclass correlation coefficient for interobserver variation was considered average/good to excellent[27] demonstrating adequate agreements. Results suggest that the counts made by observer and control observers may vary significantly in relation to actual age, but that this variation is not predominantly due to inconsistencies among observers.

When the sample is divided into images of grades 1 and 2, contrary to expectations, results show that the less positive correlation between known and estimated ages is associated with the higher quality (grade 1) images ((r) = 0.59), and not to the lower quality (grade 2) images ((r) = 0.79). Thus, the quality of the images cannot be considered the main criterion to explain inaccurate age estimates (Figs 1 and 2). A similar finding was also reported in another study.[15]

The sample split into individuals younger and older than 50 years also shows a remarkable decrease of the correlation (0.51 and 0.03, respectively) between known age and estimated age, suggesting that the technique is less effective at older ages, as reported by other authors.[7-8, 24-25] Apparently, periodontal problems are the major source of error of estimated ages. The correlation for the entire sample ((r) = 0.59, p<0.01) increases if teeth with periodontal problems are excluded ((r) = 0.74, p<0.01) and decreases significantly when only teeth with periodontal problems are considered ((r) = 0.06). The same applies to the mean errors in years (Tables 1 and 2). These findings also agree with other studies,[14, 16] but when the actual ages of individuals with periodontal problems (n=12) are analyzed, it is noticed that all of them are 50 years old or more. This fact, coupled with the decrease of accuracy of the technique with increasing age,[7-8, 24-25] may have contributed to the extremely high mean errors found. Moreover, any previous periodontal treatment (such as dental scaling) may have caused the removal of cementum, impairing age estimates. Pathological alterations of cementum in periodontitis may compromise the results for this group: the spatial arrangement of collagen fibers can be changed,[28] or their destruction may result in thinner cementum layers[29] and, consequently, less LC.

Subjects 2, 3 and 8 had cementum thickness measurements that were lower than might be expected. However, these three suffered from diabetis (condition recorded when the tooth was donated, but not disclosed to the observer until after the age estimation was undertaken). These smaller average thicknesses for diabetic patients agree with the findings of other authors.[30]
Results for cementum thickness suggest that a correlation exists between cementum thickness and actual age, as it also does between LC and actual age, however, this correlation may decrease if individuals have periodontal problems, diabetes and older ages.

The teeth of individuals 9, 10 and 19 had highly overestimated ages (mean errors of 11.7, 10.6 and 10.4 years, respectively). These teeth were periodontally sound third molars and were extracted because of caries or impaction. Because third molars may have significant variation in time of eruption, root/crown morphology and maybe quality and quantity of LC, an analysis excluding these teeth was made. Without these three outliers, the correlation for periodontally sound teeth rises to (r) = 0.80 (p <0.01). This finding further strengthens the hypothesis that periodontally sound teeth can have their ages estimated from LC.

In this study, sample size was small due to difficulties in finding available independent collectors of teeth (to avoid counting bias due to the observer and control observers having contact with the donors). Short period of time granted to access the dental clinics (two days a week, from October to December, 2009) also hampered collection of a large number of teeth. Although pilot studies for slide preparation were conducted in our research group, this technique is not widely applied in Brazilian forensic human dental examination and research. Therefore, the authors faced many histotechnical difficulties as this is the first validation of the LC technique in the country. In addition, counts were made on one microscopic slide, by one observer at one occasion. This analysis design can be up to 799% less efficient than counting eight slides by one observer at one occasion.[17] However, the preliminary results suggest a significant correlation between estimated and known age for periodontally sound teeth and a weak correlation for age estimates of periodontally diseased teeth, agreeing with previous studies carried out on larger samples in other countries.

CONCLUSIONS

- Estimating age by counting the LC added to the tooth´s mean eruption age can be a reliable method for teeth without periodontal diseases, with a mean error of 1.6 years for the sample. When examining teeth with periodontal diseases, the results provide an underestimation of age, with an average error of 22.6 years for the sample. The correlation for the entire sample ((r) = 0.58, p <0.001) was moderate, but weaker for teeth with periodontal problems ((r) = 0.06) and stronger ((r) = 0.74, p<0.01) for periodontally sound teeth.
- A similar moderate correlation between cementum thickness and known age was found ((r)= 0.69, p<0,01), but it was lower in teeth with periodontal diseases ((r)= 0.25), and higher in teeth without periodontal problems ((r)= 0.76).
- The accuracy of the technique decreased with increasing age of the individuals analyzed.
- The image quality, and the intra-and inter did not provide the major sources of error.

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Table 1: *Age estimates for teeth extracted for any reason other than periodontal disease. Pearson´s correlation coefficient (r) = 0.74, p <0.01*

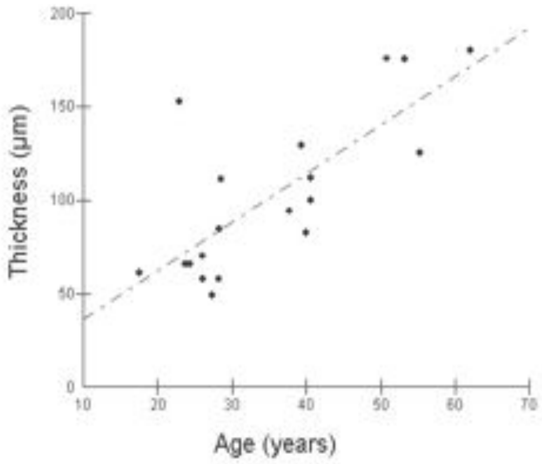
Individual	Tooth	Known age: years	Estimated age: years	Reason for extraction	Image grade
1	25	40.6	39.1	a	2
	14	40.6	35.8	a	2
5	47	26.1	24.8	c	1
	46	26.1	27.1	c	2
6	23	23.7	21.3	d	1
7	35	55.2	42	a	1
9	48	17.6	29.3	e	1
10	18	27.4	38	a	1
12	17	37.8	43.1	a	1
13	24	39.3	35.1	a	2
14	24	28.2	28.1	a	1
15	13	53.2	44	a	1
16	43	28.5	33.8	c	1
17	38	24.3	27.6	d	1
18	26	62.1	40.5	a	2
19	18	22.9	33.3	a	1
21	16	40	25.8	a	1
22	23	50.8	46.3	a	1
23	25	28.3	29.5	a	1
average	-	*35.4*	*33.8*		

Table 2: *Age estimates for all teeth extracted because of irreversible loss of periodontal attachment (reason for extraction b). Pearson´s correlation coefficient (r) = 0.06.*

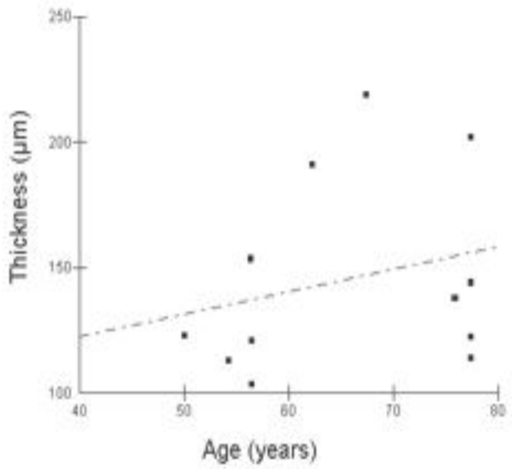
Individual	**Tooth**	**Known age: years**	**Estimated age: years**	**Image grade**
2	33	77.3	45.8	2
	32	77.3	39.1	3
	31	77.3	41.8	2
	43	77.3	55.5	1
3	22	56.4	33.5	2
	23	56.4	24	2
4	34	67.3	50.5	1
8	11	75.8	26.8	1
11	38	62.2	63.3	2
20	14	54.2	47	2
24	16	56.3	45.5	3
25	27	50	43.1	2
average	-	*65.7*	*43.1*	

Table 3: *Age estimates for observer (O) and control observers A and B (COA and COB, respectively)*

Individual	**Known age: years**	**O**	**COA**	**COB**	**Individual**	**Known age: years**	**O**	**COA**	**COB**
1	40.6	39.1	37.1	43.5	*11*	62.2	63.3	42.3	70.3
	40.6	35.8	35.8	37.1	*12*	37.8	43.1	45.8	44.1
2	77.3	45.8	41.8	48.1	*13*	39.3	35.1	35.1	35.1
	77.3	39.1	26.8	40.1	*14*	28.2	28.1	27.5	26.8
	77.3	41.8	41.8	36.1	*15*	53.2	44	34	57
	77.3	55.5	52.5	63.5	*16*	28.5	33.8	44.1	44.8
3	56.4	33.5	36.1	38.5	*17*	24.3	27.6	31	29
	56.4	24	30.6	26	*18*	62.1	40.5	30.5	41.5
4	67.3	50.5	48.8	57.5	*19*	22.9	33.3	33	40.3
5	26.1	24.8	27.1	32.1	*20*	54.2	47	50	53.6
	26.1	27.1	27.5	31.1	*21*	40	25.8	28.8	29.8
6	23.7	21.3	36	47	*22*	50.8	46.3	42.6	57
7	55.2	42	47.6	47.3	*23*	28.3	29.5	35.5	37.5
8	75.8	26.8	27.5	30.1	*24*	56.3	45.5	42.5	51.1
9	17.6	29.3	32.6	36.3	25	50	43.1	43.1	45.1
10	27.4	38	39.6	41.6	*average*	*47.1*	*37.4*	*37.3*	*42.5*



Graphic 1: *(upper) - Cementum thickness (µm) and actual age, teeth extracted for non-periodontal reasons (n = 19). Pearson´s correlation coefficient (r) = 0.75, p <0.01*



Graphic 2: *(below) - Cementum thickness (µm) and actual age, for teeth extracted for periodontal reasons (n = 12). Pearson´s correlation coefficient (r) = 0.25*

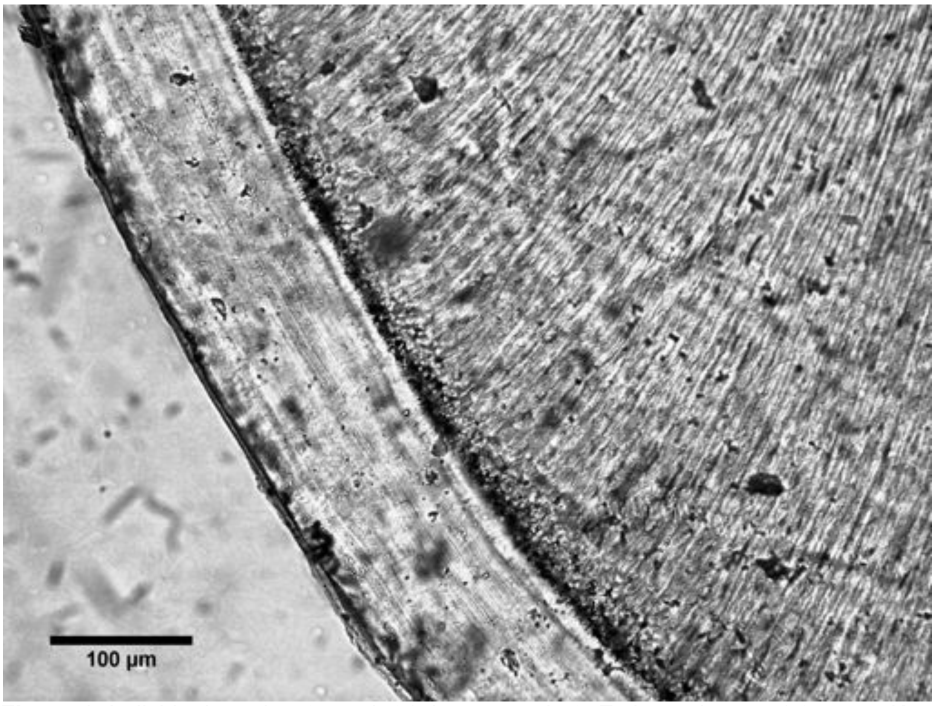


Figure 1: *Individual number 2, tooth 31, image grade 2. Estimated age: 41.8 years. Known age: 77.3 years*

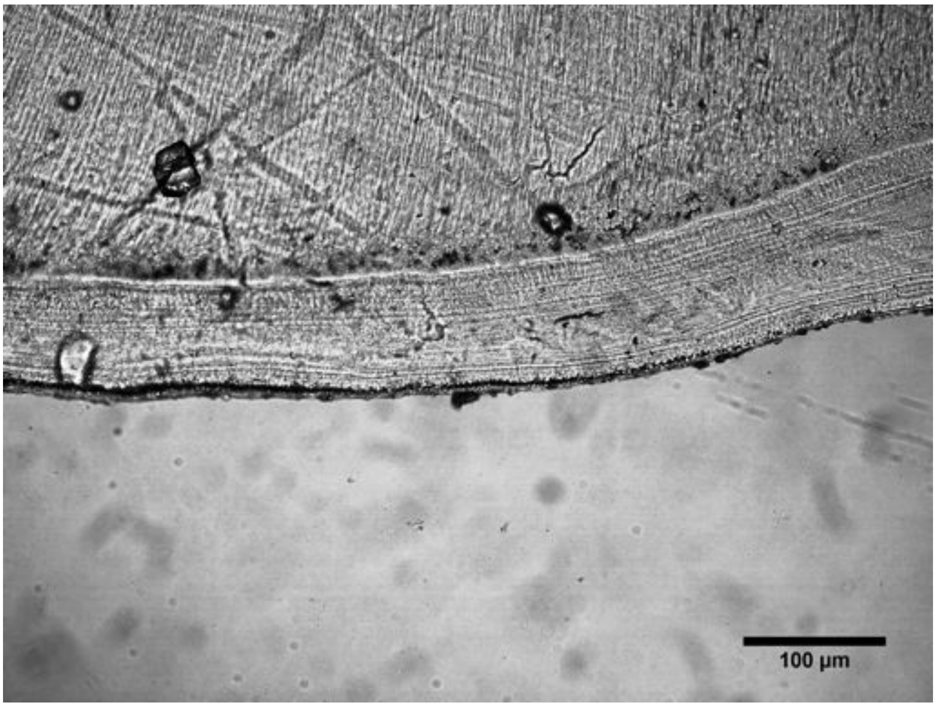


Figure 2: *Individual number 22, tooth 13, image grade 1. Estimated age: 46.3 years. Known age: 50.8 years*

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