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Forensic determination of dental age by cementum thickness of human teeth

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ABSTRACT

The purpose of this study was to assess the correlation between the known chronological age and the dental cementum thickness (DCT) in male and female subjects in different age groups. **Material and methods:** The study sample consisted of 57 donor teeth of both sexes. Teeth were classified by donors' sex and divided into three age groups: 10-19, 30-39 and 60-69 years. Tooth roots were cut with transverse ground sections in the apical, middle, and cervical thirds. DCT measurements were made on photomicrographs of light microscope. The correlation between DCT and the chronological age was calculated using the Spearman correlation coefficient. **Results:** A positive correlation was found ($r=0.47$, $p < 0.001$) between DCT and age of the donor. DCT decreased from apical to cervical ground section (median [IQR] apical section 216.72 [128.25-375.00] μm , middle section 158.44 [87.66-284.90] μm ; cervical section 96.60 [70.05-165.59] μm). DCT variability was influenced by sex, number of tooth roots and the condition of the tooth crown. The influence differed depending on the location of the section, being most prominent cervically. **Conclusion:** The present study showed correlation of DCT with age, with significant influence of sex, number of tooth roots, condition of the tooth crown and location of the root section.

INTRODUCTION

Age estimation is one of the key factors in identifying a person in both forensic research and clinical, anthropological and archaeological research of people and human remains. Although influenced by various environmental factors after death, skeletal and dental remains are the best preserved tissues of the human body. Age estimation is important for medico-legal reasons; therefore the accuracy of estimation is of particular importance for the purposes of forensic analysis.¹

Teeth, based on their life-changing morphology, can be a valid source of information about a person's age.²⁻⁴

So far, various methods have been used in humans, some of which are invasive and some non-invasive. Invasive methods result in tooth destruction, but enable greater precision because they provide detailed insight into individual parts of the structure and morphology of teeth and allow checking the accuracy and validity of non-invasive methods. One of the first methods for determining dental age in adults based on life changes on teeth dates to the 1950s, and is named as the Gustafson method, after its author.⁶ It is based on the evaluation of six parameters in the ground section: attrition,

loss of periodontal attachment, secondary dentine, cementum apposition at the root apex, root resorption at the apex and dentine translucency.

None of the dental age estimation methods in adults has withstood the test of time due to problems related to the methodology such as: insufficient sample size, narrow age range of donors whose teeth were considered, type and condition of teeth.

All methods involving the assessment of dental age, which include markers measured or assessed on the tooth crown, have the disadvantage that the tooth crown, due to exposure in the oral cavity, is the most damaged part of the tooth.⁶ Age estimation by occlusal tooth wear may be inaccurate due to various factors such as: diet type, bruxism, occupation, lateralization, masticatory forces, socio-economic status, oral hygiene, etc.⁷

It seems that the analysis of dental cementum could provide an answer to the existing shortcomings or further direct new research.

During root development, acellular cementum is deposited on the dentine of the tooth root, and from the moment the tooth comes into function, i.e. in occlusal contact with the antagonist tooth, cellular cementum is deposited throughout lifetime. Its deposition is related to age and strength of masticatory forces.^{2,3,8-15} Since it is evident that the thickness of dental cementum increases with age, dental cementum can be a valid indicator in the assessment of dental age.

It is known that the crown of the tooth wears out during lifetime. By recession of the epithelial attachment, a part of the root becomes a part of the physiological crown, while the deposition of cellular cementum compensates for the length of the root in the alveolus.

The purpose of this study was to assess the correlation of the dental cementum thickness with the chronological age of subjects, tooth donors of both sexes in different age groups.

MATERIAL AND METHODS

Sample

The sample of this pilot study consisted of 57 donor teeth of both sexes. Teeth extracted because of periodontal disease or orthodontic and prosthetic reasons were used in the research, with a strict indication from a specialist. The teeth were collected by colleagues from the clinics of the School of Dental Medicine and from several private practices. The age of the donors at the

time of tooth extraction ranged from 10 to 69 years. The teeth were divided into three groups according to the chronological age of the donor: 10 - 19, 30 - 39 and 60 - 69 years, and they were classified according to sex. The exclusion criteria were teeth with root lesions.

Each tooth was embedded in a quick-setting autoacrylate (Presi, France) and the roots were cut with transverse ground sections on the ISOMET 1000 precision cutter in the apical, middle and cervical third, using a 7 cm diameter diamond circular saw. Six transverse ground sections of the root were made on each tooth: 2 sections on the cervical part of the tooth root, 2 sections in the middle third of the tooth root, and 2 sections on the apical part of the tooth root. The thickness of each section ranged from 0.3 to 0.5 mm. The total sample consisted of 342 ground sections.

Horizontal root sections were visualized and analyzed using the Olympus model CX43 / CX33 light microscope (Olympus Corp. Tokyo, Japan), and the cementum thickness was measured on photomicrographs using the Olympus EP50 digital camera, Version: V3_20190202 under 4x magnification (Olympus Corp. Tokyo, Japan).

The measurement was performed at 4 measuring points on each of the ground sections (points a, b, c, d), clockwise, Figures 1-4.

Statistical analysis

Normality of the distributions was tested using the Kolmogorov-Smirnov test. The differences between sub-groups were tested using the chi-square test or Fisher exact test for categorical variables, and for continuous variables using the Student t-test or the Mann-Whitney U test (when comparing two sub-groups), or using the analysis of variance (ANOVA) or the Kruskal-Wallis ANOVA (when comparing multiple sub-groups), depending on distributions. For comparison of paired samples, the Friedman test was used. The association between the thickness of the cementum and the chronological age of donors was calculated using the Spearman's correlation coefficient. Predictability was calculated using models in analysis of covariance (ANCOVA).

Statistical significance for all tests was set at $p < 0.05$, with corrections for multiple comparisons. Statistical analyses were conducted using statistical software packages STATISTICA version 12 (StatSoft, Inc. (2013) and MedCalc® version 19.8 (MedCalc Software Ltd, Ostend, Belgium; <https://www.medcalc.org>; 2021).

Figure 1 Section lines and measurement points scheme

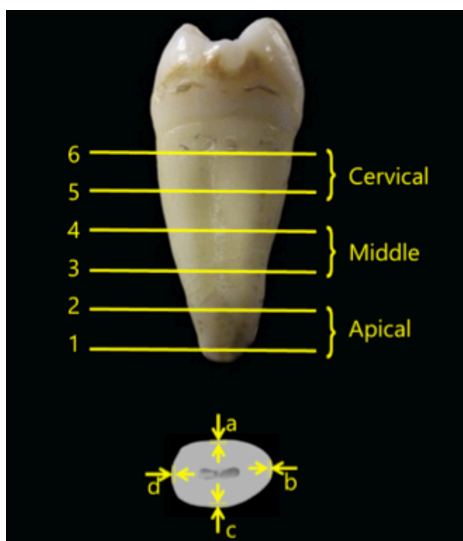


Figure 3 Measurement of cementum thickness at 1st ground section

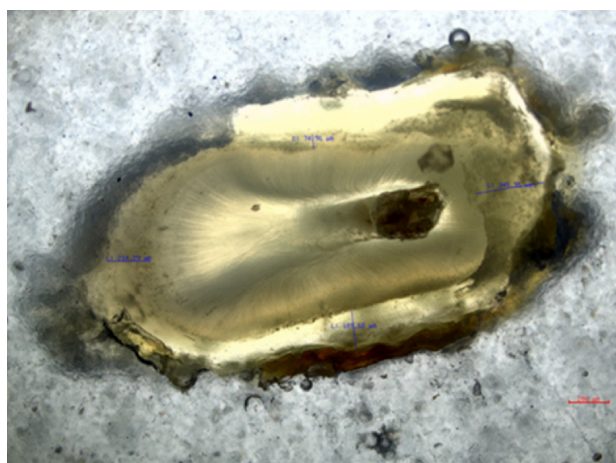
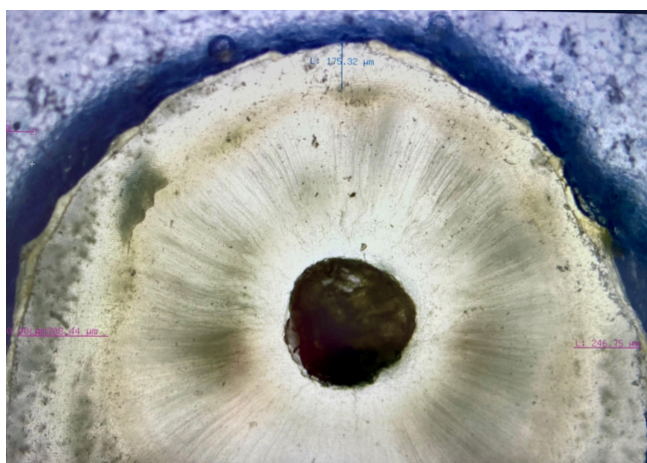


Figure 2 Cellular (C) and acellular (A) cementum, light microscope



Figure 4 Measurement of cementum thickness at 3rd (middle) ground section



RESULTS

A total of 57 teeth from the same number of individual donors were analyzed, of which 39 (68.4%) teeth belonged to the female group. Donors ranged in age between 10 and 68 years, divided into 3 age groups (10-19 years, 30-39 years and 60-69 years). The characteristics of the donors and the teeth are shown in Table 1. The thickness of the cementum decreased from the apical to the cervical level (median [IQR] apical section 217 [128-375] μm ; middle section 158 [88-285] μm ; cervical section 97 [70-166] μm). Table 2 shows the average cementum thicknesses (median [IQR]) according to age groups, sex, number of tooth roots, and tooth crown integrity. The average (median [IQR]) cementum thicknesses were 95 [58-154] μm in the 10-19 age group, 232 [184-332] μm in the 30-39 age group, and 172 [136- 284] μm in

the 60-69 age group, and they differed significantly in age groups with respect to the section site (apical, middle, cervical; $p < 0.001$, the Friedman test). They also differed significantly by age for each section site ($p < 0.001$, the Kruskal-Wallis test) (Table 2 and Figure 5 A-C). The cementum thickness has been found to be affected by sex (male teeth had a greater cementum thickness, median [IQR], 189 [104-297] μm vs. 157 [101-281] μm ; Table 2), number of tooth roots (teeth with 2 or more roots had a greater cementum thickness, median [IQR], 186 [95-263] μm vs. 145 [100-262] μm ; Table 2), and tooth crown condition (teeth with a destroyed crown had a two times greater cementum thickness compared to those with normal crown, median [IQR], 320 [117-468] μm vs. 160 [97-256] μm ; Table 2).

The influence of these factors (sex, number of tooth roots, and condition of tooth crown) on cementum thickness depends on the site of the measurement (section site). Although it did not reach statistical significance, it was greatest in the cervical region, Table 2. These factors were not evenly distributed by age categories, Table 3.

A positive correlation was found between cementum thickness and known chronological age of the donor ($r = 0.41$, $p = 0.002$ for cervical cementum thickness; $r = 0.45$, $p < 0.001$ for middle and 0.47 , $p < 0.001$ for apical and average cementum

thickness).

The analysis of covariance, which included sex, number of tooth roots, condition of tooth crown and thickness of cementum, determined that the apical thickness of cementum ($F = 6.527$, $p = 0.014$) with the interaction of sex and number of tooth roots was the best determinant of donor age ($F = 5.376$, $p = 0.025$) with the coefficient of determination $R^2 = 0.321$ ($F = 4.626$, $p = 0.002$ for the model). The coefficient of determination for middle, cervical and average cementum thickness was 0.314 , 0.267 and 0.317 , respectively.

Table 1 Donors and teeth characteristics

Variable	
Subject age at extraction, median age (range)	33 (10-68) years
10-19 years (%)	22 (38.6)
30-39 years (%)	15 (26.3)
60-69 years (%)	20 (35.1)
Sex, female (%)	39 (68.4)
Number of roots	
1 root (%)	34 (59.6)
>1 roots (%)	23 (40.4)
Crown intact (%)	52 (91.2)
Apical cement thickness, median (IQR)	217 (128-381) μm
Middle cement thickness, median (IQR)	158 (88-286) μm
Cervical cement thickness, median (IQR)	97 (69-166) μm
Average cement thickness, median (IQR)	171 (100-281)

IQR – interquartile range

Figure 5 Apical (A), middle (B) and cervical (C) cementum thickness according to the age groups; median, 95% CI for the median, 25-75 percentiles, 10-90 percentiles, and range is presented

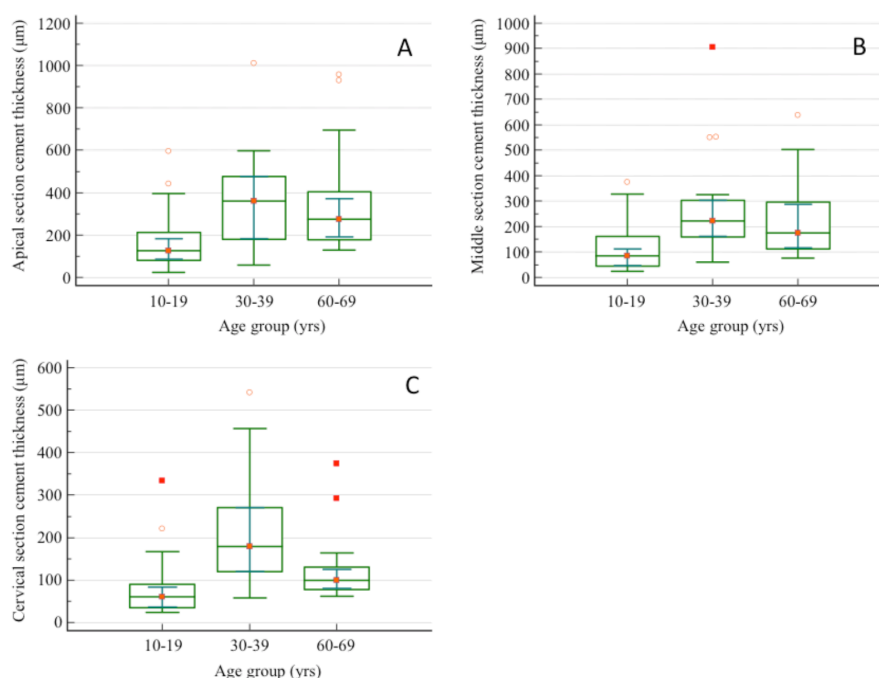


Table 2. Cementum thickness of different sections according to age groups, sex, root number and crown status

Categories	Cement thickness, median (IQR)				p-value (Friedman test)
	Apical	Middle	Cervical	Average	
Age					
10-19 years (%)	127 (81-214)	86 (45-161)	60 (36-90)	95 (58-154)	<0.001
30-39 years (%)	362 (181-478)	222 (161-304)	179 (120-271)	232 (193-326)	<0.001
60-69 years (%)	274 (179-405)	174 (112-296)	99 (78-131)	172 (136-284)	<0.001
p-value (Kruskal-Wallis test)	<0.001	<0.001	<0.001	<0.001	
Sex					
Female	196 (128-362)	155 (91-288)	90 (71-137)	157 (101-281)	<0.001
Male	251 (142-478)	163 (88-222)	136 (51-225)	189 (104-297)	<0.001
p-value (Mann-Whitney test)	0.286	0.813	0.343	0.520	
Number of roots					
1 root (%)	214 (128-361)	133 (88-287)	90 (65-124)	145 (100-262)	<0.001
>1 roots (%)	207 (122-366)	170 (85-239)	128 (71-226)	186 (95-263)	<0.001
p-value (Mann-Whitney test)	0.945	0.492	0.159	0.536	
Crown status					
Intact	203 (126-363)	155 (88-237)	91 (70-149)	160 (97-256)	<0.001
Destructed	411 (232-508)	274 (110-550)	223 (54-309)	320 (117-468)	<0.001
p-value (Mann-Whitney test)	0.082	0.153	0.185	0.096	

IQR – interquartile range

Table 3 Sex, root number and crown status distribution according to age groups

Variable	Age group			p-value (chi-square test)
	10-19 years	30-39 years	60-69 years	
Sex				
Female	14 (63.6%)	8 (53.3%)	17 (85.0%)	0.076
Male	8 (36.4%)	7 (46.7%)	3 (15.0%)	
Number of roots				
1 root (%)	14 (63.6%)	3 (20.0%)	18 (90.0%)	<0.001
>1 roots (%)	8 (36.4%)	12 (80.0%)	2 (10.0%)	
Crown status				
Intact	20 (90.9%)	11 (73.3%)	20 (100%)	0.049
Destructed	2 (9.1%)	4 (26.7%)	0 (0%)	

DISCUSSION

In this study, we wanted to assess the association between the known chronological age and DCT in male and female subjects in different age groups, regarding additional factors that could affect DCT. The results showed a moderate degree of correlation of the chronological age with cementum thickness. They also showed that there is a sex difference in cementum thickness. In the teeth of male donors, a greater thickness of cementum was measured in all age groups and at all root levels, however being the most pronounced in the cervical sections. We also found a significant difference in cementum thickness with respect to the distance from the tooth apex: cementum was thickest at the apex, and the thickness decreased toward the cervical portion of the tooth root. The number of tooth roots also influenced the thickness of the dental cementum.

It is interesting to note that the condition of the dental crown also affected the thickness of dental cementum. A significantly greater thickness of cementum was measured in teeth with a destroyed dental crown.

Previous research done by invasive methods was limited by sample size, selection of only one group of teeth, and the narrow age range. Therefore, positive correlations and statistical significance of the obtained data offer very limited practical application in the identification of human remains. Most of the research is based on the comparison of dentine and cementum as the preferred tissue for reliable data, the comparison of the measurement of the deposited cementum thickness and the number of incremental lines, as well as the comparison of vertical and horizontal sections in cement.¹⁰⁻¹⁹

In addition, most of the available scientific papers took into consideration exclusively intact teeth, mostly only one group. There were some exceptions that corroborate our data. For instance, the work of Pinchi et al.²⁰ found that upper teeth measurements offer more reliable data. However, the presented data for different groups of teeth (incisors, canines, etc.) offers small samples over a wide age range. On the other hand, they argue the advantage of using simpler analytical methods. Since in forensic and anthropological cases we cannot choose which teeth to have as material for identification and assessment of age, we conducted research on a sample of teeth of both sexes, all categories,

without excluding teeth with partially destroyed tooth crown. Other authors, like Dias et al.²¹ also explored the influence of dental pathology, specifically periodontal disease on the association between cementum thickness and age. They showed that this association is significantly disturbed by the presence of periodontal disease (leading to a prediction error of 22.6 years).

In this research, we decided to increase the accuracy of assessment through a previously unused method of analyzing the measurement of DCT on multiple horizontal sections, including all categories of teeth, both sexes, in a wide age range from 10 to 68 years. Horizontal sections were used to preserve the samples as based on the published experience of other authors. Amariti et al. state that longitudinal sections broke many teeth and introduced a lot of artefacts thus significantly affecting the results.²² By selecting the age groups for this study, we wanted to compare the teeth of the youngest group of donors (10-19 yrs.), in which the acellular cementum is dominant, middle age (30-39 yrs.), in which the deposit of cellular cementum is expected to be significant, as well as the influence of external factors on the tooth, and older age (60-69 yrs.), where the apical resorption of the cementum is expected to have already begun.

Shruuthi et al.²³ conducted a study to assess dental age in a population of South India, on a sample of 150 teeth, the age of the donors at the time of extraction 15-75 years, comparing two assessment methods: dentine translucency and deposited cementum thickness in single-rooted teeth (incisors and canines). The results showed that both methods were reliable in middle-aged subjects, while great deviations were obtained in younger and elderly people. Therefore, the authors suggest using the quantitative measurement of cementum deposition in younger specimens, and before the onset of the translucent dentine formation phase. The largest difference in reliability and accuracy was measured in subjects older than 60 years. Estimation of age by DCT, however, proved to be marginally better because dental cementum in the area of the tooth root was minimally affected by resorption processes.

Mohan et al.²⁴ examined age on a sample of 20 teeth using the number of incremental lines. They found a significant correlation between an individual's actual age and estimated age using

the dental cement annulations method, but in a narrow age range.

Mani-Caplazi et al.²⁵ investigated the causes of irregular incremental lines in cementum, which may be affected by pregnancy or some diseases, indicating a lack of reliability in the method of using incremental lines in age assessment.

Mallar et al.²⁶ compared longitudinal and transverse sections in a sample of 50 subjects of both sexes. Although the sample size of 25 male teeth and 25 female teeth is sufficient for such a study, the method of using only one (middle) section is not sufficiently reliable. The results showed greater reliability in longitudinal sections, but only in donor specimens younger than 30 years.

Swetha et al.²⁷ examined the correlation of known age and number of incremental lines in a sample of 80 single-rooted teeth, ranging in age from 22-60 years, to assess which tooth is the most reliable indicator of age. The study used longitudinal sections and the formula stating that the estimated age is equal to sum of the number of incremental lines and eruption age of the particular tooth. A positive correlation was obtained between known and estimated age, with a mean deviation of ± 2 years. Lieberman⁴ pointed out in his research that cementum is the best hard dental tissue for age assessment because it is continuously deposited, unlike other dental tissues, and due to no blood flow, it is less susceptible to remodelling and resorption processes.

CONCLUSIONS

There was a gradual increase in the thickness of the cementum commensurate with ageing, hence

the amount of cementum can be used as an indicator of age in post-mortem forensic analyses. The thickness of the cementum is greater in males, particularly in the cervical third of the tooth root. The obtained quantitative values of dental cementum thickness yielded results that can be used in developing a new formula for dental age estimation in adults. More reliable results will be obtained by conducting a study with a larger sample, which is in progress.

Limitations of the study

Relatively small sample, uneven distribution of factors affecting the thickness of cementum by age groups, absence of comparing different methods of cutting or determining the thickness of cementum (different microscopes), absence of comparing different methods of fitting the samples into the cutting mixture, without the data on the method of extraction and storage of teeth after extraction and previous manipulations related to the root of the tooth are the main limitations of our study.

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Ethical codex: This research was approved by the Ethics Committee of the School of Dental Medicine, University of Zagreb at the 18th regular session held on 04.06.2020, decision number: 05-PA-30-XVIII-6/2020.

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