



Cementum Annulations as a Tool for Age Estimation In Forensic Odontology

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ABSTRACT-

Introduction: Estimating age at death is a key element in the process of human identification of remains of the Skeleton. It is a vital process of forensic aspect of dentistry. Cementum annulation technique is one of the key techniques to estimate the age of a person after death. Recent research shows that this technique of tooth cementum annulations (TCA) may be used more reliably than any other morphological or histological traits of adult skeleton for age estimation.

Aim: The purpose of this study is to estimate the age of the subject using tooth cementum annulation method by incremental lines present in the cementum.

Materials and Methods: The study consisted of the sample of twenty teeth that had been extracted from the patients ranging in the age of 20-70 years. Longitudinal ground sections of each tooth were cut and was examined under the microscope. The images were then zoomed / magnified on the computer and the number of dark lines were counted. The mid root region was selected for counting the annulations. The counted cementum lines for each tooth were then added to the eruption age of the tooth to estimate the age of the patient.

Results: There was found a strong positive correlation between the estimated age and the calculated age of the person when examined under the microscope. This proves that cementum annulations can help give an accurate estimation of the persons age irrespective of the sex, age, periodontal disease etc.

Conclusion: With this study we were able to conclude that counting the incremental lines can improve the accuracy of age estimation, in turn helping the process of forensic profiling of the subject.

Introduction

Identification of a person living or dead is of paramount importance in routine forensic odontology. The determination of age plays an important role in forensic medicine not only in identification of bodies but also in connection with various crimes.¹ According to Keiser-Neilson in 1970, forensic odontology is a branch of medicine that deals with the proper handling and examination of dental evidence with proper evaluation and presentation, in the interest of justice.²

In the past numerous researchers have proposed various age estimation techniques using different skeletal and dental tissues. The accuracy of these age estimation techniques is much greater in children and adolescents as compared to adults due to the presence of a multitude of developing teeth.³

Age estimation in adults has been studied and teeth are very important tools for the identification of the age of an

individual as they differ from bones in their biological properties and function and are well preserved.²

The cementum is a connective tissue that surrounds the tooth root and slowly gets deposited throughout life. Cementum remains stable throughout an individual's life as the racemization of aspartic acid in cementum continues constantly.⁴ The age of a person can be assessed based on

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its apposition and by counting the number of annulations between the cementum dentine junction and periodontal ligament.⁵

The assessment of age at death in historic skeletons has traditionally employed the method of counting cemental annulations, known as the tooth cementum annulation (TCA) method. This technique has now been broadened to encompass forensic cases.⁶ This technique of Tooth cementum annulations was first observed by Scheffer and Laws, who observed alternating light and dark bands in the teeth of sea mammals.⁷ Unlike other dental hard tissues, cementum is less exposed to outer environmental surroundings, because of its unique position in the alveolar process, thereby increasing its accuracy towards estimating the age of an individual.⁸ These incremental lines can be best observed in the acellular cementum which are present in the cervical and middle third region of the root.

Thus, the purpose of this study is to estimate the accuracy of age of a person by studying the number of incremental lines present in the cementum of their tooth.

Materials And Methods

This is a pilot study performed in the Department of Oral Pathology and Microbiology over two months. Institutional ethical clearance was obtained before starting the study. The study sample consisted of 20 teeth extracted from patients aged between 20-70 years. Samples were collected from the Department of Oral and Maxillofacial Surgery for one week and the teeth that met the inclusion and exclusion criteria were included with no randomization.

Inclusion criteria

- Single-rooted teeth
- Whole root was present
- Only teeth from patients age between 20-70 years

Exclusion criteria

- Non-functional teeth
- Teeth with excessive like attrition, hypercementosis etc.

After obtaining the teeth, they were fixed in formalin and then washed in running water before they were ground to obtain thin sections approximately 100-150 microns in thickness. The sectioning was done using an Arkansas grinding stone. Sectioning was done longitudinally and hence the incremental lines were parallel to the long axis of the tooth. During sectioning or grinding, some of the sections broke completely or parts of the cementum broke

away; such sections were not included in the study. Only intact tooth sections were included in the sample. Only 20 sections of 20 teeth (one section per tooth) were included in the final sample.

After sectioning, the specimens were again washed under running water and immersed in xylene to clear off the debris from the sectioned specimen. The sections were then mounted on a glass slide using DPX mounting medium. The slide was later observed under a light microscope. Photomicrographs of each slide were taken and recorded. The areas where the incremental lines were continuous to count irrespective of whether acellular and cellular cementum were considered. The age was obtained by adding the number of counted incremental lines to the age of the eruption of the tooth by using the following formula [Age of the patient = Number of incremental lines + Age of tooth eruption]

The observations were recorded on an excel sheet and Karl-Pearson correlation coefficient was used for drawing inferences.

Results

The actual and the estimated ages of the persons based on light microscopy are shown in Table 1.

The Karl-Pearson correlation coefficient showed a strong positive correlation between the estimated age and calculated age. (Table 2)

Discussion

In forensic science, the estimation of a person's age can aid investigators in identifying individuals involved in crimes or major disasters.² Age estimation is crucial not only for contemporary cases but also for analyzing ancient skeletal remains in paleodemographical studies.³ Various methods for age estimation rely on the structured changes occurring during tooth formation or eruption. The thickness of cementum, due to its continuous apposition, holds potential as a tissue for age estimation.⁹

The etiology of cementum bands remains unclear, with theories suggesting factors such as the degree of mineralization, collagen fiber orientation, and seasonal variations.⁵ Cementum, composed of 40% collagen and 60% mineral, is favored by many researchers for determining chronological age.¹⁰ Ground sections for tooth

**Table 1:** Cemental annulations, estimated age and actual age of each tooth sample

Specimen No.	Tooth No.	Number of Annulations	Eruption Age (years)	Estimated Age= No. of Incremental Lines + Eruption Age	Actual Age (Years)
1.	24	25	9	34	37
2.	44	32	9	41	43
3.	35	41	10	51	56
4.	23	36	11	59	63
5.	31	38	7	45	51
6.	11	45	7	52	57
7.	13	28	11	39	43
8.	21	36	7	43	52
9.	24	28	10	38	39
10.	45	23	11	34	38
11.	25	15	10	25	28
12.	24	16	9	25	28
13.	23	45	11	56	60
14.	15	16	10	26	25
15.	25	12	10	22	23
16.	24	28	9	37	40
17.	24	25	9	34	38
18.	35	12	10	22	24
19.	33	30	11	41	53
20.	44	31	9	40	47

Table 2: Correlation between estimated age and actual age

	Mean	Std. Deviation	r	p-value
Estimated age	38.200 0	10.92848	0.978	0.001
Actual age	42.250 0	12.45994		

$p \leq 0.05$ – Significant, CI = 95 %

cementum annulations (TCA) can be prepared longitudinally or in cross sections, and the technique for preparing and isolating cementum for TCA is very technique sensitive.^{11,12}

Protocols and techniques established by Wittwer-Backofen and Buba are widely employed in TCA estimation.¹³ Cementum annulations have been utilized for age determination in various animals such as including otter, moose, squirrel, bat and bear.¹⁰ Factors like hormonal fluctuations, nutritional status, and ecological conditions can induce variations in cementogenesis, influencing the appearance of annulations.

The first layer of acellular cementum is produced before the tooth erupts, and the further layers are added during and

after the eruption of the tooth. Lieberman and Schroder gave the biological explanation for these alternating layers.¹⁴ The dark lines are referred to as the incremental lines and the cementum between each two lines have been referred to as incremental bands. It was observed that each pair of the lines corresponded to one year of life and that it all constituted a biological record which could be helpful in the age estimation of the person.¹ Incremental lines, formed before tooth eruption, constitute a biological record that corresponds to the person's age.⁶ The influence of the parathyroid hormone in incremental line formation as well as difficulty in counting the lines during application of this technique may lead to creating a bias.⁷

Studies have explored optimal sections for counting



annulations, with varying preferences for longitudinal or cross sections. In this study we used the mid-root area of teeth for counting annulations. Most specimens showed that the difference between actual age and calculated age was in the range of 3-4 years, making TCA a fairly reliable guide for age determination in adults. Similar findings matched those published by Stott et al.¹⁵ who carried out a study on teeth extracted from cadavers. Wittwer-Backofen et al.¹⁶ made use of digital software for counting cemental annulations and obtained a similar difference of 2-3 years in their actual and predicted ages.

Avadhani et al.¹⁰ had shown that transverse sections were better for counting cemental annulations, although they also reported that they were unable to count annulations in around 24% of the total sample. In our study, we used longitudinal sections single-rooted teeth and although we rejected some ground sections as they got fractured our sample size did not show any attrition due to inability to count cemental annulations.

To summarize, although there are limitations in calculating the age through TCA due to variables such as susceptibility to periodontal diseases, systemic conditions, and other age-related alterations that may affect the accuracy of dental age prediction. The present study highlights that with meticulous processing and precise sectioning, along with the application of light microscopy and photography, the enumeration of cemental annulations emerges as a viable approach for determining age.

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