Research Article

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Cemental Annulation Rings: Role in Age Estimation

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ABSTRACT

Background: In the past various forensic odontology methods have been employed for the age estimation of an individual. Tooth is one of the best methods for the same when the remains of the individual were destroyed due to natural or unnatural reasons. In the past, it had been proved to be valuable. Cementum (alternate light and dark bands) a part of tooth has been founded proportionate to the age of the patient in number. Hence the present study was planned to determine the role of tooth cementum annulation in the age estimation.

Materials and Methods: The sample size for the present study was thirty extracted teeth. Sociodemographic details as well as the chief complaint of the patients were recorded. Formalin was used to preserve the teeth overnight. Before the sectioning, teeth were washed under the tap water. After the sectioning, the photographs of the extracted teeth were taken to count the lines under 10 × objective of light microscope. Observer was blinded regarding the age of the patient during analysis so that chances of bias can be avoided. Statistical analysis was done using SPPS version 22.

Results: It was found that mean incremental lines and age of the tooth eruption are significant ($p<0.01$) in predicting the ages. There was no significant difference was detected over the observed and predicted ages.

Conclusion: The results of the present study concluded that quantification of cementum annuli is a moderately reliable means which can be used for age estimation in humans.

Keywords: Tooth, age estimation, cementum annulation, forensic odontology.

INTRODUCTION

Forensic odontology can be defined as a branch of dentistry, which deals with the proper handling and examination of dental evidence and with the proper evaluation, and presentation of dental findings in the interest of dentist[1]. It is often essential in the fields of physical anthropology, wildlife management, and forensic medicine to determine the age of various animal species, including man. One method of recent interest for this is the enumeration of annulations present in the cementum of permanent teeth[2]. Age estimation of the individual by the tooth is beneficial when all his other remains are ruined due to natural or unnatural reasons. Due to variation in properties of teeth and bones in term of function as well as preservation, teeth served as better tool for age estimation[3].

Anatomical root is sheltered by cementum which during a person's life remains steady. This is because of the fact that in cementum racemization reaction of aspartic acid in a stable way[4]. This method is becoming more widely used and accepted because age of the individual was reported proportionate to number of alternate light and dark bands in the cementum. Therefore the aim of the

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present study was to evaluate the person's age by counting the incremental lines present in the cementum with the help of cementum annulation method.

MATERIALS AND METHODS

The present prospective study was conducted in the Department of oral pathology and microbiology, Anil Neerukonda Institute of Dental Sciences, Visakhapatnam. The research was planned for a period of month i.e. between February to March 2019. The research was started after the institution gave the ethical clearance.

Sample: The present study comprised of 30 freshly extracted teeth from the patients aged 14-40 years. The teeth extracted were premolars and molars from maxilla as well as mandible. The sample size was depended on the availability, due to which randomization was not done. The teeth were selected based on the following inclusion and exclusion criteria:

Inclusion criteria: Teeth extracted for orthodontic purpose or due to carious involvement, patients subjected for orthodontic extraction and extraction due to caries without periodontal involvement were included in the study.

Exclusion criteria: Periodontally compromised teeth, teeth subjected to attrition, abrasion, and erosion were excluded from the study.

In the present research, selection bias was avoided by sticking to the inclusion criteria. 10% formalin was used to preserve the extracted overnight and then thoroughly washed under running water. The teeth were then stranded to acquire thin section of 80 to 120 μm thickness. The teeth were sectioned longitudinally so that the incremental lines were parallel to the long axis of the tooth. After sectioning, the samples were again washed under running water. Each section was finally cleared in xylene for 2 min to clear off all the debris and then mounted using Permount on a properly labelled glass slide and allowed to dry for 4 h. The slide was later viewed under light microscope with a 10×objective. Photomicrographs of each prepared slide were taken and recorded and areas where incremental lines were continuous and easy to count irrespective of whether cellular or acellular cementum was considered. Photomicrographs were taken by using Cannon IXUS 190, digital camera and the lens was magnified five times. The photographs were further processed with Adobe Photoshop CS7 software, and counting was done on enlarged photographs by two separate observers (figure 1). Both cellular and acellular cementums were viewed for counting the lines. Dark and light incremental lines of cementum were not clearly viewed in all the sections. Only the dark lines suitable for counting were selected. The observers were blinded to the age of the individual and the average of the result was obtained. The age of the patient was thus obtained by adding the number of counted incremental lines to the age of the eruption of the tooth as follows: Age of the patient = Number of incremental lines + the age of the tooth eruption.

Statistical analysis: Data so collected was tabulated in an excel sheet, under the guidance of statistician. Data was analyzed using IBM SPSS. Statistics Windows, Version 22.0. (Armonk, NY: IBM Corp) for the generation of descriptive and inferential statistics. Statistical analysis was done using regression slope, correlation table, and the level of significance was set at $p<0.05$.

RESULTS

Tooth eruption age is presented in table 1, graph 1, for example, dental age of 10 had a frequency of 53.33%. Individual's age was calculated by adding the total number of incremental lines from each slide to the eruption age of that tooth (table 1). Table 2 shows the frequency of age in years where age 14 had a frequency of 2 which made up to 6.67% of the total number of teeth and so on. Table 3 represents the mean frequency of incremental lines where the average incremental line of 1.5 had an occurrence of 3.33% and so on. Mean incremental lines and the dental age correspond to the actual age of the patient are signified in Table 4 with the help of regression equation. Using the regression equation, the age is predicted corresponding to the mean incremental lines and age of the tooth eruption [Table 4]. Considering the significance values, we observe that mean incremental lines and age of the tooth eruption are significant ($p<0.01$) in predicting the ages [Tables 5]. Hence, no significant difference was detected over the observed and predicted ages.

Table 1: Tooth eruption age.

Age of tooth eruption	N	%
10	16	53.33
11	8	26.67
11.5	2	6.67
19.5	4	13.33
Total	30	100

Table 2: Frequency of age in years.

Age (years)	N	%
14	2	6.67
15	1	3.33
16	6	20.00
17	3	10.00
18	4	13.33
19	3	10.00
20	2	6.67
21	3	10.00
22	1	3.33
24	1	3.33
28	1	3.33
29	1	3.33
37	1	3.33
40	1	3.33

Table 3: Mean incremental lines among the study sample.

Mean incremental lines	N	%
1.5	1	3.33
3	1	3.33
4	2	6.67
5	3	10.00
6	2	6.67
6.5	3	10.00
7	5	16.67
8	2	6.67
8.5	3	10.00
9.5	3	10.00
10.5	3	10.00
22	2	6.67

Table 4: Prediction of age using regression equation.

Observed Age	Mean incremental lines	Age of the tooth eruption	Predicted age
14	4	10	14.63
14	3	10	13.22
15	1.5	11	15.38
16	4	10	15.11
16	6	10	16.79
16	5	11	16.68
16	5	11	16.69
16	6.5	10	17.02
16	5	10	16.72
17	4	19.5	15.92
17	6	10	17.82
17	6.5	11	18.02
18	6.5	10	18.98
18	7	10	19.06
18	8.5	11	19.13
18	8	10	19.08
19	7	19.5	19.72
19	8	10	19.80
19	7	10	19.64
20	10.5	11.5	21.09
20	9.5	10	20.86
21	7	10	21.12
21	9.5	19.5	24.89
21	10.5	11	21.19
22	7	11	22.99
24	8.5	10	25.11
28	9.5	19.5	28.74
29	10.5	10	30.06
37	22	11	38.11
40	22	11.5	40.69

Table 5: Correlation between age and dependent variables of mean incremental lines.

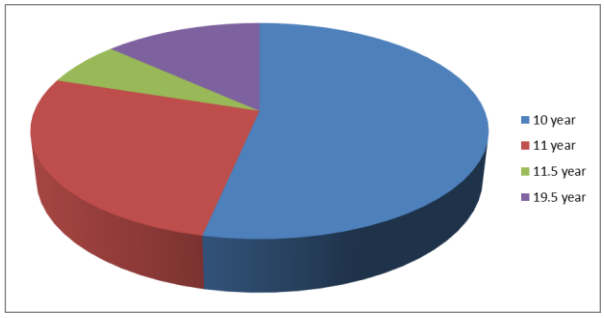
Variables	Age (in years)	
	R	p value
Mean incremental lines	0.84	<0.01*
Age of the tooth eruption	0.27	0.19

*: statistically significant

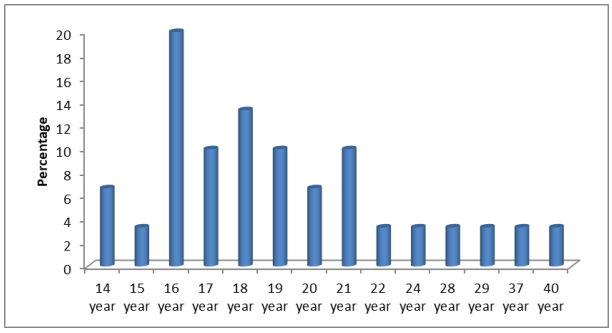
DISCUSSION

There are numerous methods available for the assessment age of an individual such as dental or skeletal morphology and laboratory methods such as racemization of aspartic acid in dentin or tooth enamel or radiocarbon dating of tooth enamel[5]. In age assessment, importance of tooth was realized when all other leftovers of a human are damaged. Teeth are usually well protected from fire and mechanical trauma and are highly resistant to post-mortem destruction and decomposition[6]. Cementum is the calcified tissue that surrounds the

root portion of dentin and it forms the attachment site for the periodontal fibres that link the tooth to the alveolar bone. In cementum formation, hypermineralized layers of extracellular matrix alternate with less mineralized layers. The first layer of acellular cementum is produced before the tooth erupts, and further layers are added during and after eruption[7]. A biological explanation for these alternating layers was given by Lieberman and Schroder. They suggested that the dark lines were the stop phases of mineralization during continual growth of the fibroblasts, which led to a change in mineral crystal orientation. This pattern is visible under the microscope as a series of alternating light and dark lines or bands. The dark lines have been referred to as incremental lines and the cementum between each two lines has been referred to as incremental bands. It was shown that each pair of lines corresponded to one year of life and that it constituted a biological record that could be used to estimate the age of an individual[8].



Graph 1: Age of eruption.



Graph 2: Age frequency in years

The first use of cementum in human age estimation began with measurements of width of the total cementum layer, rather than the number of incremental lines[9]. Stott et al., first used Tooth Cemental Annulations (TCA), as an age estimation method in humans and concluded that these annulations which were counted from a photograph provided a close estimate of the actual age of the individual from which the tooth was extracted[10].

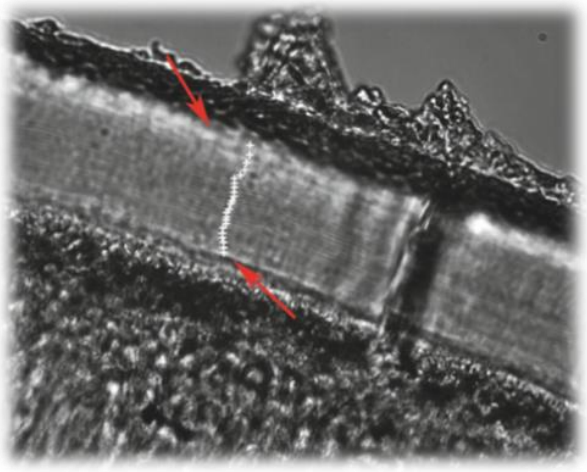


Fig 1: Tooth cemental annulations image with counting overlay marking each cementum layer, the eruption layer as borderline between dentine and cementum, and the outer root surface layers.

In the present study, Pearson's correlation coefficient between estimated age from cemental lines and actual age was found to be strongly positive i.e. significant at a level of 0.01. Thus, counting of cemental annulations may be an accurate method for age estimation in human beings. Similar results were reported by Condon et al[11] in their study. They reported a high correlation between adjusted counts of cementum layers (i.e., the number of layers added to the age of tooth eruption) and age at extraction or death. Aggarwal P et al[12], found a fairly strong positive correlation between the two variables of estimated age which was calculated from cemental lines and the actual age. According to Avadhani et al[13], in 18 of 19 specimens, the estimated age varied from chronological age by about 2-3 years. The reliability of this method was found to be 94.73 %. N. Mohan et al[6] also revealed that a correlation exists between the actual age of the individual and the estimated age using tooth cementum annulation method.

Conflicting results had been reported in the literature regarding the reliability of cementum's alternate light and dark band in the estimation of the individual age. For example, in a study by Mallar et al[14], it was found that cementum lines are most correctly calculated in the middle third of the tooth

root and, though the cross sections are easier to count, more reliable results are determined from longitudinal sections. According to Kaur et al[15] phase contrast microscope followed by polarized and light microscope were the best options to count

found the observer error and tooth variability in this technique for age estimation and concluded that premolars are the favoured tooth with least inter-observer error. Also, by increasing the number of slides per tooth, tooth variance can be minimized.

The present study was conducted on only thirty samples and in future studies with larger sample must be conducted determine the accuracy in age estimation. Application of this method with accurate results in periodontally compromised teeth has not been proven.

Conclusion: Cemental annulations, which indeed are a reflection of age, are generally difficult to count, especially when they are numerous and close together. Tooth cementum annulation technique can be applied in forensic odontology to estimate the individual's age. Numerous studies have also shown that cementum annulation method can be used in determining age in anthropological study of ancient population. This study did not allow us to conduct a detailed investigation on the impact of different individual parameters such as sex and tooth position, Further studies comparing the diverse methods for reviewing the cementum annulation can establish the practicality of this technique in age estimation.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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