Quantitative analysis of dental age estimation by incremental line of cementum

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INTRODUCTION
Age is one of the indispensable factors in forming the uniqueness of the individual. Age estimation is a technique implemented by many archaeologists, anthropologists and many forensic scientists. There are numerous methods such as odontometric, histological, radiographies and biochemical methods for dental age estimation, but none of the methods are precise for dental age estimation; each method will have some limitations and they have not given satisfactory results.[1] The concept of age estimation changes era in forensic odontology. In the identification of dead bodies, age estimation becomes obligatory if there are no antemortem details available and if there is a need for renovation of particular profile.

Aim: This study aims to examine the correlation between age and number of incremental lines in human dental cementum among single-rooted teeth (incisors and canines) and to assess the best tooth to estimate age group by studying cementum under phase-contrast microscope and to assess the use of cementum in age estimation.

Materials and Methods: The present study was carried out in the Department of Oral Pathology and Microbiology. A sample of eighty single-rooted undecalcified longitudinal ground sections is prepared from freshly collected teeth with age noted separately and observed under phase-contrast microscope, photographed and counted. Counting the number of alternating dark and light lines of the cementum and adding them to the average age at which the analyzed tooth erupts provided an estimate of the chronological age of the individual.

Results: The results obtained in the present study were statistically significant and positive correlation was observed between the actual age and the estimated age. The number of incremental lines was found to be gradually increased with increase in age of the individual. On an average, ±2 years of age difference were observed in our study on comparing the actual age with estimated age.

Conclusion: Hence, the incremental lines of the cementum were found to be gradually increased with increase in age and hence can be used as one of the adjuvant tools in dental age estimation.

Keywords: Actual age, cementum, estimated age, incremental lines, phase-contrast microscopy

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Moreover, estimation of age can be done invaluable archaeological skeletal material cutting back to hundreds of centuries. Tooth development begins in intrauterine life, and the individual age will start at the age of 21 to 60 years. Young individuals will show strong correlation of chronological age with estimated age.

In a developing country like India, a huge numeral of persons is uneducated and do not have awareness or archives of their birth date which is obligatory in cases of illegal acts, documentation, jurisdictional punishment, agreement, rape, unlawful abortion, service, accomplishment of majority, abduction and in cases of prostitution. In all of these circumstances, estimation can be used as an adjuvant tool in approximating the age of the particular individual. Estimation of age is a compulsory for entrance in schools, joining services, at the time of retirement and during the improvement of pensions to the old. Hence, the technical determination of age plays an important role.

Further age can also be designed from the advent of ossification centers and their unions during skeletal advance. Even though forensic odontology is a quite trivial sphere, it has been exploited for countless years particularly in forming individuality. The chief reported crime in the antiquity was cracked when bite marks were revealed in the remnants of the prohibited fruit in the Orchard of Eden and recognized as those belonging to Adam and Eve. Teeth are one of the structure will with stand with external factors such as temperature, decomposition. Dental cementum is the mineralized, a vascular dental tissue covers the anatomical roots of human teeth. The mineralized substance of the cementum continuously deposited on the root surface. Dental cementum is one of the tooth structures we can use for dental age estimation. Continue apposition of cementum will cause deposition of cementum and resting of cementum will appear as incremental deposition of cementum. These incremental lines can be used for dental age estimation. Counting the alternative dark and white bands of incremental lines plus average age at which tooth erupts provides an estimation of an individual. Estimation of dental age using incremental lines of cementum first described by Scheffer in 1950. Stott et al., in 1982 done a study to correlation between incremental lines of cementum with dental age, he found that estimated age is close to actual age.

There are different techniques used for assessment of incremental lines of cementum, but a decreased accuracy of the technique in more advanced age. The purpose of this study was to evaluate the correlation between the number of incremental cemental annulation with knowing age group using phase-contrast microscope.

MATERIALS AND METHODS

Eighty freshly extracted single-rooted teeth were collected from eighty individuals with known age. Teeth were collected from the Department of Oral and Maxillofacial surgery, SIBAR institute of Dental Sciences, Guntur, South India. All teeth were extracted for discretionary dental treatment. The inclusion criteria were extracted due to periodontitis and trauma and exclusion criteria were teeth extracted due to caries, grossly decayed teeth, deciduous teeth and teeth with cementum associate pathologies are excluded from the study. After collection of teeth kept in formalin and teeth were grouped according to age grouped into I–VIII, each tooth is cut into thin sections using a diamond tipped disc and sectioning was done long axis of the tooth. The teeth were then ground on Arkansas stone with water to 80 µm thicknesses. The sections were cleared with xylene and mounted using dibutyl phthalate in xylene mounting media on glass slides. In each section, the area at the junction of the cervical and middle third of the root where a cellular cementum is present is considered for counting the incremental lines of the cementum. Digital images for the incremental lines were taken for every section using phase-contrast microscope under ×20 magnification. Photomicrographs were visualized on the computer and the cemental line were counted with the help of image analysis software image express pro version 6.0 attached to the Olympus phase-contrast microscope (BX 51M) with Jenoptik digital CCD camera. Alternate dark and white bands are observed in photomicrographs in the present study [Figure 1]. Estimated age of the teeth was calculated by using the formula given by Stott in 1982.

Incremental lines are calculated with formula: Estimated age = Number of incremental lines + eruption age of that particular tooth.

Figure 1: Incremental lines of cementum using phase-contrast microscope (×200)
Statistical analysis
Number of incremental lines were counted, and number was entered in excel sheet and data, statistical analysis were performed using Statistical Software Package for the Social Sciences (SPSS) 20.0 version. Comparison of actual age and estimated age was done by paired t-test. Intragroup correlations were carried out for each group by applying Karl Pearson’s correlation coefficient.

RESULTS

The study sample consists of eighty single rooted teeth, which were divided into eight groups according to the age with ten teeth in each group [Table 1]. Association between age and number of incremental lines was calculated using descriptive statistical analysis and the results revealed that at the age of the individual increased, the number of incremental lines was found to be increased significantly [Table 2]. The actual age was minimum of 22 years and maximum of 60 years. Comparison of mean, among actual age with an estimated age by using paired t-test. The mean and standard deviation of actual age is 40.63 ± 11.55. The mean and standard deviation of estimated age is 40.19 ± 11.67. Hence, a positive correlation was observed between actual age and estimated age [Table 3]. When compared with mean of actual age and estimated age the observations showed statistically not significant with \( P \geq 0.05 \), whereas correlation between actual age with number of incremental lines are statistically significant \( (P \leq 0.05) \) [Table 4]. Comparison between estimated age with number of incremental lines by using Karl Pearson’s correlation coefficient observation shown statistically significant \( (P \leq 0.05) \), but when correlated between estimated age and eruption age, it is not statistically significant \( (P \geq 0.05) \) [Table 4].

Multiple regression analysis of actual age was done with number of incremental lines and the eruption age with independent variables. Intercept value was statistically significant \( (P \leq 0.05) \). Independent variables using a number of incremental lines were found to be statistically significant \( (P \leq 0.05) \) [Table 5].

DISCUSSION

Dental age estimation is very important aspect in forensic investigations and application ranges from comparison of antemortem with postmortem identification to estimating age in childrens and adults. [9] Jones et al, in 1974 mentioned cementum as a hard avascular connective tissue that covers the roots of the teeth. The primary function of the cementum is to anchor the tooth root to the gum maintaining the crown in position for effective occlusion. [9] As the position of cementum is intermediary, it forms the interface between root dentin and periodontal ligament. One of the main functions of cementum is to anchor the principal collagen fibers of the periodontal ligament to the root surface, but it also has adaptive and reparative functions, playing a crucial role in maintaining occlusal relationships and in protecting the integrity of the root surface. [14] The unique property of cementum is that it does not undergo continuous remodeling like bone but

Table 1: Groupwise distribution of the study participants according to age

| Serial number | Groups | Age group |
|---------------|--------|-----------|
| 1             | Group I| 21-25     |
| 2             | Group II| 26-30    |
| 3             | Group III| 31-35   |
| 4             | Group IV| 36-40    |
| 5             | Group V | 41-45     |
| 6             | Group VI| 46-50    |
| 7             | Group VII| 51-55   |
| 8             | Group VIII| 56-60 |

Table 2: Descriptive statistics on groups with mean of incremental lines

| Serial number | Groups | Mean number of incremental lines |
|---------------|--------|----------------------------------|
| 1             | Group I| 11.9                             |
| 2             | Group II| 18.3                             |
| 3             | Group III| 21.5                             |
| 4             | Group IV| 24.7                             |
| 5             | Group V | 30.6                             |
| 6             | Group VI| 35.7                             |
| 7             | Group VII| 40.1                             |
| 8             | Group VIII| 46.2                             |

Table 3: Comparison of mean, among actual age with an estimated age by using a paired t-test

| Variable       | Mean±SD | t     | P   | Inference |
|----------------|---------|-------|-----|-----------|
| Actual age     | 40.63±11.55 | 1.18  | 0.24| NS        |
| Estimated age  | 40.19±11.67 |       |     |           |

SD: Standard deviation, NS: Not significant

Table 4: Correlation between actual age and estimated age with number of incremental lines and eruption age by Karl Pearson’s correlation coefficient

| Variables       | Correlation between actual age with | Correlation between estimated age with |
|-----------------|-------------------------------------|----------------------------------------|
|                 | Number of lines                     | Eruption age                            |
|                 | \( r \) \( t \) \( P \)             | \( r \) \( t \) \( P \)                |
| Number of lines | 0.9570 0.291268 0.0001*             | 0.9889 58.8279 0.0001*                 |
| Eruption age    | 0.1416 1.2633 0.2102                | 0.2034 1.8351 0.0703                   |

\* \( P \leq 0.05 \)

Table 5: Multiple regression analysis of actual age by number of incremental lines and eruption age

| Indpt variables | Estimate | SE of estimate | t     | P     |
|-----------------|----------|---------------|-------|-------|
| Intercept       | 6.1790   | 2.6326        | 2.3471| 0.0215*|
| Number of lines | 0.9636   | 0.0320        | 30.1209| 0.0001*|
| Eruption age    | 0.3943   | 0.2160        | 2.7515| 0.0074*|

\* \( P < 0.05 \), SE: Standard error

Jones et al, in 1974 mentioned cementum as a hard avascular connective tissue that covers the roots of the teeth. The primary function of the cementum is to anchor the tooth root to the gum maintaining the crown in position for effective occlusion. As the position of cementum is intermediary, it forms the interface between root dentin and periodontal ligament. One of the main functions of cementum is to anchor the principal collagen fibers of the periodontal ligament to the root surface, but it also has adaptive and reparative functions, playing a crucial role in maintaining occlusal relationships and in protecting the integrity of the root surface. The unique property of cementum is that it does not undergo continuous remodeling like bone but
continues to grow in thickness throughout life. Unlike dentin and enamel, where there are clear differences in the proteins present in these tissues and the factors regulating their functions when compared with bone, cementum has not demonstrated to express specific proteins and factors in common with bone and to be developmentally controlled by similar factors.[15] The importance of incremental lines of cementum was highlighted by explaining the properties that make archeologists and wildlife biologists to be helpful in many ways and also enlightened the seasonal variations of incremental lines of the cementum.[16] When sectioned and analyzed under microscope, they appear as alternating translucent and opaque bands.

Zander and Hurzeler in 1958 done a study to correlate between cementum annulations with dental age, in their study they observed that straight line relationship between actual age with incremental lines of cementum and also said that when the dental age increases the number of incremental lines also increases.[17] Selukar et al. in 2002 done a study on cemental apposition with dental age. They found that significant positive correlation between age of individual and cementum apposition. They concluded that when age advances the cemental apposition and number of incremental lines are increased.[18] We have observed in our study that incremental lines of cementum with dental age estimation, we observed that positive correlation between incremental lines of cementum with estimated age, the mean difference was ±2–3 years. When the age advances the number of incremental lines of cementum were increased, these observations were similar to Selukar et al., in 2002. Gupta et al. in 2014 done a study to investigate the role of cementum as an aid for age estimation and to get the correlation between secondary cementum and age of the individuals. A strong positive correlation was found between the estimated age, which was calculated by using cemental lines and actual age. They found that the thickness of the cementum was increased with increase in age. So they concluded that countable cemental annulations are present in human teeth and quantification of cementum annuli is a moderately reliable means which can be used for age estimation in humans.[19] in our study when correlated between estimated age and actual age we observed that positive correlation in both the genders and estimated age and actual age.

**CONCLUSION**

Age estimation from human teeth is well recognized. Dissimilar methods and abundant procedures have been established for age estimation, every single one signifying specific accuracy, exactness and reliability. In all circumstances, reproducible and consistent estimation effects are possible when the suitable methods are appropriately applied and used. Miscalculation is present in every single case. Hence, the forensic odontologist should follow diverse techniques and accomplish repetitive depths and calculations in order to come to a consistent conclusion. When compared with other methods used in forensic odontology for dental age estimation, counting cemental annulations is easy to perform, not technique sensitive, less time taking and economically feasible. Moreover, cementum does not undergo much pathological changes when compared with other structures of the teeth. Hence, in conclusion, incremental lines of the cementum can be used as an adjuvant tool in dental age estimation.

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**Conflicts of interest**

There are no conflicts of interest.

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