

Age Estimation with Cemental Incremental Lines in Normal And Periodontally Diseased Teeth Using Phase Contrast Microscope

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Abstract

Background: Forensic age estimation defines an expertise in forensic medicine, which aims to define in the most accurate way to determine the unknown chronological age of the person involved in judicial or legal proceedings. Dental cementum is a vital tissue which demonstrates continuous apposition throughout the life of the tooth. Literature studies have revealed that tooth cemental annulations(TCA) would serve as a reliable tool in establishing the age of an individual. The use of specialized microscopic methods are been employed to enhance the assessment of the cemental annulations. The study aims at assessment and evaluation of cemental annulations in normal and periodontally diseased extracted teeth using phase contrast microscopic method. **Methodology:** A total of 60 teeth were included in the study and out of which thirty teeth were normal and 30 were periodontally involved teeth respectively. Longitudinal ground sections were prepared using micromotor and diamond disc. Sections were mounted and observed under phase contrast microscope. Measurements were made using an image analyzer software. The total width of cementum was divided by the distance between two incremental lines. The eruption age of the tooth was then added to this to obtain the chronologic age for each individual. The results of the study showed no significant deviation of estimated age from the actual age in both periodontally sound and periodontally diseased teeth.

Conclusion: The study concludes that the use of phase-contrast microscopy in conjunction with image enhancement procedures improves the accuracy of age estimation and may serve as a reliable aid in forensic identification.

Key Words: Age estimation, cementum, forensic odontology, microscope

Introduction

Human individuality is the trademark of civilization, and the identification of this individualism plays an important role in human identification. In this modern speedy epoch, several incidents are taking place, varying from natural calamities to unwanted disasters. Identification of the victims of such disaster is of paramount importance in forensic medicine.¹ Forensic odontology is considered as reliable and trustworthy for identification of the deceased as well as living

individuals. In the oro-facial region tooth serves as the best source in forensic science. Teeth are one of the hard tissues which undergo least amount of degenerative changes when exposed to environmental factors.²

Age estimation plays a vital role in the recognition of an individual. Assessment of age is not only done in the deceased but it may be many a times necessary to determine the age of living persons who are either unwilling or unable to reveal their identity.³ Teeth undergo structural changes throughout the life, these

changes form the core essence for age estimation. Cementum is a hard tissue of tooth root which is deposited regularly throughout the life of an individual, even when individual growth is completed.⁴ This regular deposition of cementum occurs in a rhythmic pattern ensuing the appearance of dark and light bands. One pair of dark and light band represents one increment which is deposited annually.⁵ The counting of cemental annulations i.e. the Tooth Cementum Annulation(TCA) method was applicable to historical skeletons and cremations to assess the age at death and has now been extended to forensic cases.

Counting cemental annulations require histological examination of thin tooth sections. Previous studies have demonstrated the use of various microscopic methods and reported that incremental lines are best viewed through phase contrast microscopy. Technical improvements and new technologies to help differentiate the lines have been proposed⁶, but decreased accuracy of the technique in more advanced ages and the influence of periodontal diseases are still factors that require better understanding.

Few studies have applied TCA method on periodontally affected teeth and demonstrated varying results. Therefore, the purpose of this study was to evaluate the correlation between the number of incremental lines in cementum and age of the individual with the aid of phase contrast microscope; and also, to analyze the influence of periodontal health on the age estimates.

Methodology

The present study was carried out in the Department of Oral Pathology and Microbiology. The study was conducted on extracted teeth from individuals of age ranging from 20 - 50 years. Extracted teeth were obtained from the Department of Oral and maxillofacial Surgery. The study samples comprised of a total 60 teeth, of which 30 were sound teeth and remaining 30 were periodontally diseased teeth, respectively. The chronological age of the patients from whom the teeth were extracted was recorded. The teeth selected for the

study were fulfilling the following criteria:

Inclusion criteria:

Teeth extracted for the following reasons were included in the study: -

- Orthodontic treatment
- Prosthetic treatment
- Impactions
- Periodontal disease

Exclusion criteria:

Teeth extracted for the following reasons were excluded in the study: -

- Teeth with periapical pathologies
- Root canal treated teeth
- Teeth with history of trauma

Preparation of ground sections:

The extracted teeth were preserved in 10% buffered formalin. Formalin fixed teeth were rinsed in water for several hours. Teeth were then treated with sodium hypochlorite and hydrogen peroxide to remove the organic debris and stains. Longitudinal sections of teeth were prepared using low speed micromotor and diamond disc. The sections were then ground using arkansas stone. The sections were then mounted using DPX.

Microscopic analysis:

These sections were examined under phase contrast microscope at a magnification of 10X, using Olympus research microscope (BX53). On microscopic examination the cementum showed alternately arranged dark band and light band. One dark band along with a light band following it was considered as one annulation.

The middle portion of the root was selected for counting the annulations.⁷ The apical part of cementum was excluded because of the increase in thickness and cellularity of cementum in this region.⁸



Figure 1: Photomicrograph of normal tooth showing measurement of total width of cementum (ground section 10x)

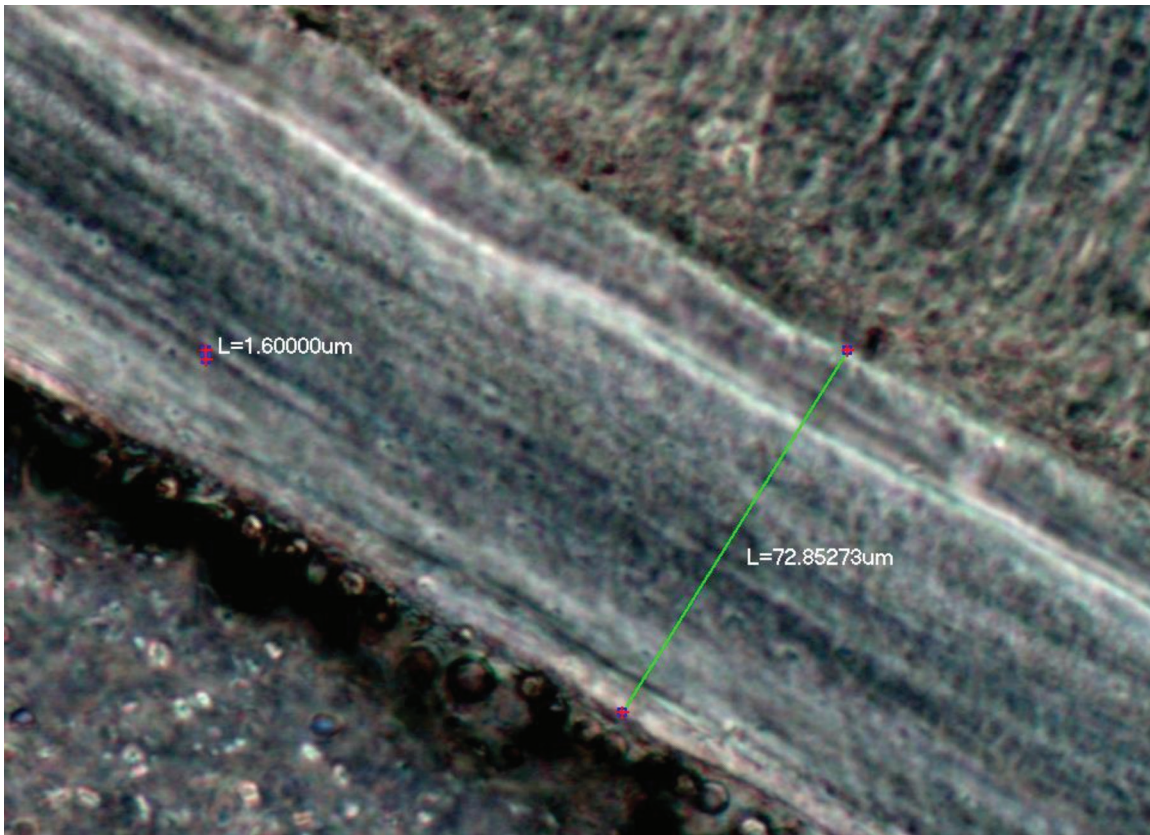


Figure 2: Photomicrograph of periodontally diseased tooth showing measurement of total width of cementum (ground section 10x)

The measurements were made for the total width of cementum and the width between two adjacent incremental lines. (Figure 1 and 2). The width of cementum was taken from the surface of cementum to the

dentinoceamental junction (DCJ) and the width between two incremental lines was taken between two adjacent lines which were easily recognizable and seemed to run approximately parallel to each other. (Figure 3 and 4).

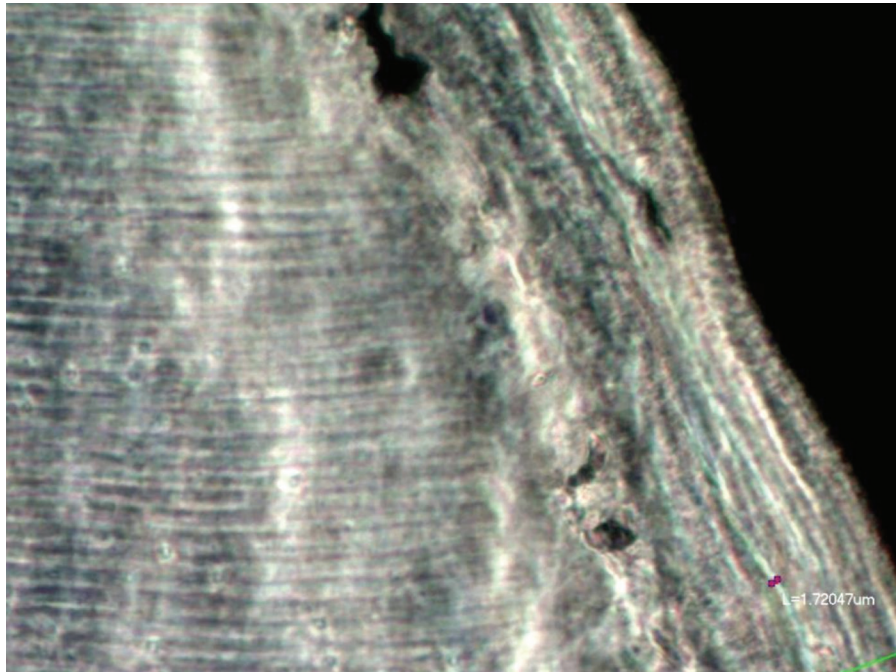


Figure 3: Photomicrograph of normal tooth showing measurement of width between two incremental lines of cementum (ground section 10x)

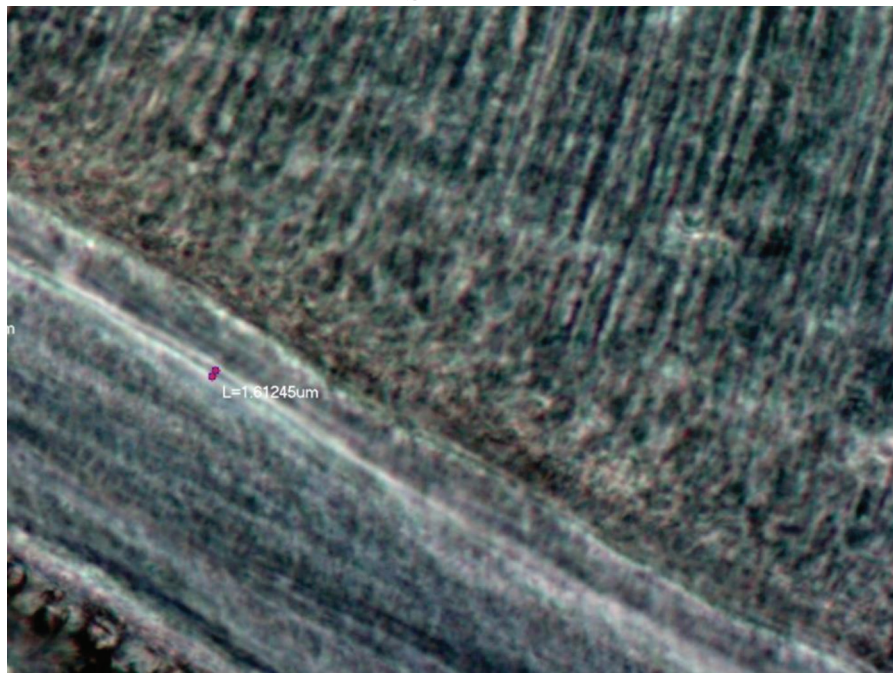


Figure 4: Photomicrograph of periodontally diseased tooth showing measurement of width between two incremental lines of cementum (ground section 10x)

The area selected for counting was photographed under 10X magnification, using a digital camera. The images were then transferred from the microscope to a computer, and counting was done with the help of image analyzer software (ScopeImage 9.0). To reduce interobserver variability, the counting of cemental lines was done by three observers. The mean value of the three observations was used for statistical evaluation.

Estimation of age:

The number of incremental lines in the total cementum width was calculated by the formula:

$$\text{Number of incremental lines (n)} = X / Y$$

where, X is the total width of cementum (from DCJ to cementum surface) and Y is the width of cementum between the two incremental lines.

The chronological age of the individual was obtained, by adding average age of eruption in years for each tooth to the counted number of incremental lines.⁹

$$\text{Estimated age (E)} = \text{number of incremental lines(n)} + \text{eruption age of tooth (t)}$$

Statistical analysis:

The data obtained was subjected to statistical analysis. Data was analyzed using Statistical Package for the Social Sciences (SPSS) Ver 23.

1. Descriptive, student paired t test was done for intragroup comparison of actual age and estimated age.

2. Regression analysis was done for reliability of the procedure of age estimation in normal and periodontally diseased teeth.

Results

Dark and light incremental bands of cementum were observed in all the longitudinally sectioned specimens. Cemental annulations in longitudinal sections of sixty teeth respectively were counted and analyzed for their correlation with actual age of the person using phase contrast microscope. The measurements of cemental annulations, calculated and the actual chronological age of individuals were tabulated for sections of normal teeth and sections of periodontally diseased teeth.

Table 1: Comparison of mean values of actual age and predicted age in both groups

Group	N	Actual Age		Estimated Age		Mean Difference in Age			
		Mean	SD	Mean	SD	Mean	SD	t value	p value
Normal Teeth	30	27.833	5.9659	28.903	6.1250	-1.07	1.7	-3.442	0.002**
Periodontally compromised teeth	30	39.367	8.4261	40.393	8.4818	-1.02	1.67	-3.354	0.002**

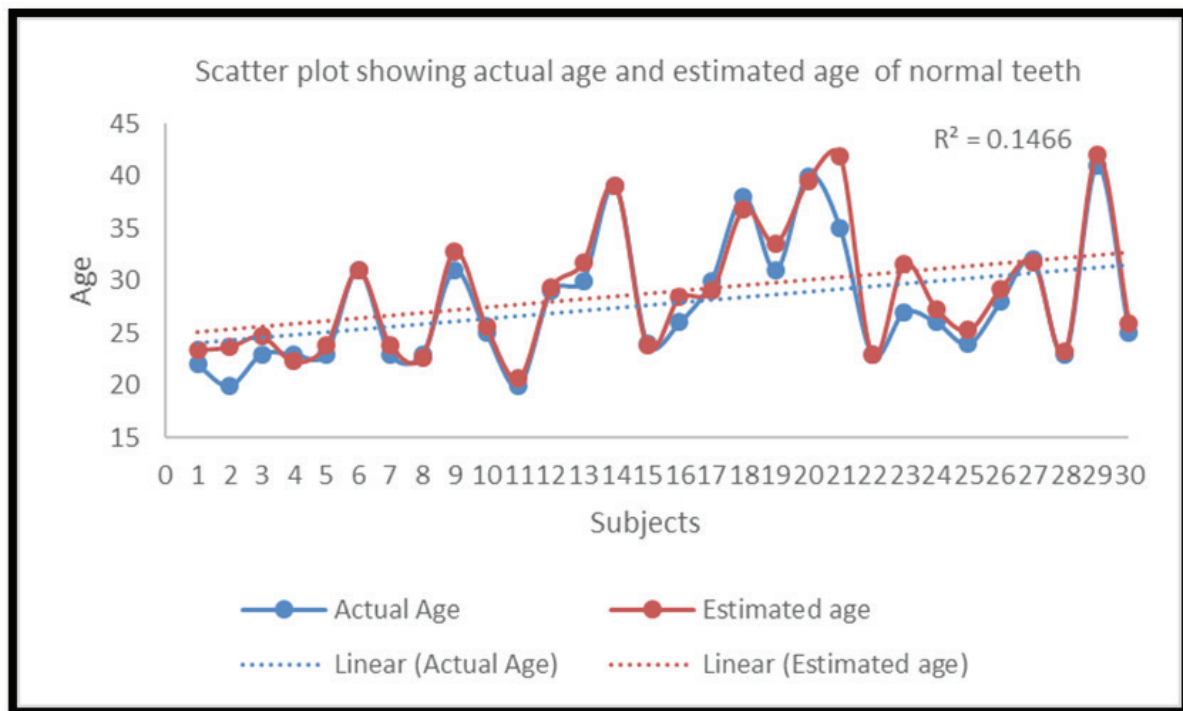
** - Statistically Highly significant (p<0.01)

The mean and SD of actual age and estimated age in both the groups are compiled in Table 1. There is statistically significant differences present in the mean values of actual age and estimated age in both the groups (p=0.002), (-) value indicates that actual age is less than the predicted age.

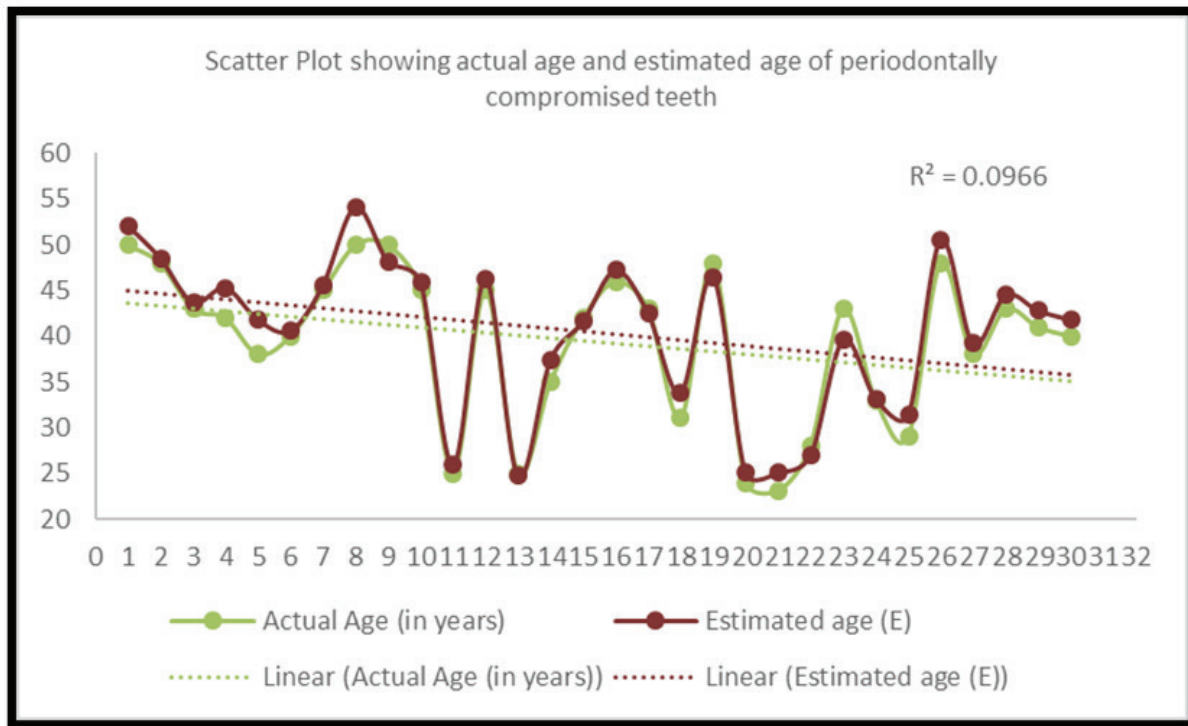
Table 2: Regression analysis for validation of the estimated age

Group	Variables	(B)	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	R square	Std Error	F value	Sig
Group 1	Intercept	0.79	1.51	0.52	0.61	-2.30	3.88	0.922	1.685	335.12	<0.001**
	Actual Age	0.94	0.05	18.31	0.00	0.83	1.04				
Group 2	Intercept	1.54	1.51	1.02	0.31	-1.55	4.64	0.961	1.702	691.91	<0.001**
	Actual Age	0.99	0.04	26.30	0.00	0.91	1.06				

** - Statistically Highly significant (p<0.01)



Graph 1: Scatter plot showing actual age and estimated age of normal teeth



Graph 2: Scatter plot showing actual age and estimated age of periodontally diseased teeth

Table 2 is a consolidated Regression analysis test output. For normal teeth, the reliability value of 0.92 which indicates the equation for age estimation is 92% accurate and the standard error of the estimate is 1.68 which indicates that out of 100 entries there is a chance of error in 2 individuals. ($p < 0.01$). For periodontally diseased teeth, the reliability value of 0.96 which indicates the equation for age estimation is 96% accurate and the standard error of the estimate is 1.70 which indicates that out of 100 entries there is a chance of error in 2 individuals ($p < 0.01$). The scatter plot revealed only minor deviations from the normal distribution and allowed regression analysis.

Regression equations:

Group 1: Normal teeth

$$\text{Estimated Age (Y)} = 0.79 + 0.94 * \text{Actual Age (X)}$$

Group 2: Periodontally diseased teeth

$$\text{Estimated Age (Y)} = 1.54 + 0.99 * \text{Actual Age (X)}$$

Discussion

Age estimation is a domain of the forensic sciences which forms an important part of every identification process, especially when information relating to the deceased is unavailable. Teeth are considered to be a source of abundant data in the process of somatic development and the vicinity in which they are formed. The periodic incremental features present in teeth provide information on the developmental rate and can be used to assess the developmental age of a tooth.¹⁰

Cementum is one such specialized calcified structure of teeth that covers the entire surface of root. Cementum is deposited in layers during and after eruption of the tooth. Continuous apposition of cementum leads to formation of two types of layers with different optical properties. Histologically, these layers are visible as alternate light and dark lines or bands.¹¹ The layered appearance is due to the structural differences in the mineral phase, an optical phenomenon that is possibly related to altered mineral crystal orientation and reflects a cyclic annual formation pattern.¹²

Each pair of these alternate bands accounts for one incremental deposit. The number of incremental deposits when added to the chronological year of eruption of the respective tooth gives the histologic age of the individual under study.⁵ Cementum comprises of a biological evidence that can be used to estimate the age of the individual.¹³ Many researchers have suggested the use of cementum of teeth for determination of human chronologic age.⁸

Cementum annulations have been studied in previous literature extensively in animals and humans.¹⁴ Several authors have studied TCA for age estimation and reported varied findings ranging from failure in applying the technique to humans, to a significantly low margin of error in age estimations for this technique.¹⁵ So, the present study was designed to evaluate the age using TCA method.

In the current study, a sample of sixty extracted teeth was included from patients with an age range between 20 and 50 years old since by the age of 20 years growth and dental development were completed as confirmed by Someda et al.¹⁶

The method of tooth sectioning is also an important consideration. Some authors prefer cross sections and others reported longitudinal sections to be appropriate, each section having some advantages and disadvantages over the other.¹⁷ Longitudinal sections allow examining the whole root surface such as advocated by Klevezal and Kleinenberg¹⁸. Stott et al.,¹⁴ and Avadhani et al.,⁸ prefer cross sections as they allow a series of observations. Maat et al.,¹⁹ recommended to cut the sections perpendicular to the exterior of root not perpendicular to the root axis. Mallar et al.,¹⁷ compared longitudinal sections with cross sections for age estimation and found that longitudinal sections were better than cross sections for estimating age.¹⁷ So, in the present study, longitudinal sections have been used to evaluate cemental annulations.

Sectioning of tooth can be done either manually by preparing ground sections on Arkansas stone or by using a hard tissue microtome. Hard tissue microtomes can be used to maintain uniform thickness of the sections.

Structures which are visible in ground sections of about 100- μ m thickness may disappear in thin sections. Use of hands in grinding was injurious to the fingers.²⁰ Although hand grinding is tedious and injurious method, some investigators prefer this and are of the opinion that it is not replaceable by any other method.¹⁷ The present study used manual sectioning.

Various authors have compared the feasibility of counting cemental annulations using different microscopes. Pundir S et al., estimated age based on TCA method using bright field, polarizing and phase contrast microscopy and suggested that the incremental lines are best viewed through phase contrast microscopy.²¹ Similar findings were suggested in other studies conducted by Bhondey A et al.,²² Gowda CBK et al.,³ and Kaur P et al.,²³ who observed that phase contrast microscopy was better than light microscopy for evaluating the cemental lines. The clarity of TCA by phase contrast microscope was explained by Sanderson who discussed how phase contrast microscope can properly distinguish between two types of layers with different optical properties. He found that phase contrast microscope depends on refraction of light by the specimen as it utilizes the difference between light rays propagating directly from the light source and light rays refracted by the specimen when light passes through it to add bright/dark contrast to images of transparent specimens and this done by the aid of a phase-contrast objective and a condenser fitted with phase contrast microscope for observations. He depicted that phase contrast microscope is suitable for viewing colorless and transparent specimens (as cementum).²⁰ So, the present work illustrated that the use of phase contrast microscopy served as a good observatory for assessing cementum annulations.

Manual counting of cemental lines is time consuming and is potentially subjective. The use of an image analyzer has got an added advantage of enhancing the view for measuring the cemental lines. In the present study, phase contrast microscopy in conjunction with image analyzer were used to enhance the cemental annulations, thereby reducing the margin of error of using simpler microscopic method.

In the present study, we used the middle third region of the root for counting cementum annulations similar to the studies conducted by Mallar et al.,¹⁷ and Aggarwal et al.⁹ In the mid-root region of a tooth, the cementum present is usually acellular and the annulations are evenly placed and less compressed than the cementum near the cemento-enamel junction (CEJ).^{11,24} Acellular cementum examination in the mid root section minimizes hindering factors such as cementocytes, has adequate thickness because of its slow and constant growth and is thus more useful in estimating age of death.²⁰

The results of our study are in consensus with Charles et al.,²⁴ Condon et al.,²⁵ Maat et al.,¹⁹ and Wittwer Backofen et al.,²⁶ who reported a well-correlated connection between cementum layering and chronological age in their studies with a significant p value of < 0.05. However, our results contradicts the studies done by Lipsinic et al.,²⁶ Lucas and Loh and Miller et al., who did not reveal a relationship between chronological age and count of tooth cementum annulations in their studies.²³

In the current research, we assessed the TCA method for age estimation in normal teeth and periodontally compromised teeth. Wittwer Backofen et al.,²⁶ used the computer software for counting the cemental annulations and found that the variation between the actual and estimated age was found to be in the range of 2-3 years. Aggarwal P et al.,⁹ in their study found a mean error of 1-2 years. In our study, the variation between the actual and estimated age was found to be 1 year in both normal and periodontally compromised teeth. The calculated probability is 0.002 for both the groups which is statistically highly significant ($p < 0.01$).

The variability in age of eruption of a certain tooth type, also the incremental line count will always lead to an age determination within a certain range, but in our study we considered the mean eruption age rather than the eruption age range. If the age range of eruption of tooth was considered it would correspond to the variation range in calculated age.

The statistics also revealed the effect of periodontal health on age estimates. The results in the present study showed that TCA is applicable to periodontally sound teeth as well as in periodontally diseased teeth. It is observed that normal teeth showed a reliability value of 92% and periodontally compromised teeth showed 96% respectively. Our study is found to be in accordance with Wittwer Backofen,²⁶ Aggarwal P⁹ and Tyagi N⁷ who concluded that the cemental annulation count did not vary in the presence of periodontal disease, but our findings contradicted the results of Dias PEM⁶, who suggested that a correlation exists between incremental cemental lines and actual age, but however, this correlation decreased if individuals have periodontal problems. Kvaal and Solheim²⁸ found that teeth extracted because of periodontal disease showed a weaker correlation with age than did the sound teeth. Kagerer and Grupe⁵ depicted that periodontally affected teeth with sufficient nutritional support of their roots showed minimal deviation of histological age from the known age.

In our study, we assessed a lower mean age (age range 20-50 years), as literature recommended an age-limited applicability of the TCA method for age estimation.⁵⁶ According to studies conducted by **Kvaal** and **Aggarwal**, in lower age groups the correlation coefficients between chronological tooth age and incremental lines were stronger than in the higher age groups.^{9,28} The accuracy of tooth cemental annulation method decreased with increasing age. Usually this was interpreted as a metabolic disorder of higher age with the influence of periodontal regression, dental caries, or other individual characters cumulating over age.²⁹ Previous studies indicated that predicted age counts for those over 55 years of age showed greater divergence from actual age.²⁶ Similar to the previous studies we have also found a stronger correlation in lower age group.

Our study sample included 3 samples of third molar teeth, 2 in normal teeth group and 1 in periodontally involved group. Deviation of calculated age from actual age was observed in these teeth. Third molars are teeth with highest variability concerning anatomy, agenesis

and age of eruption and therefore its significance as developmental marker has been questioned. Age estimation by means of third molars is limited due to its biological variance.³⁰

In the current research we also observed that there was no substantial influence of periodontal health on the estimated age. This provides a strong support for application of TCA method in archeological skeletal samples in which most individuals suffered from extreme dental disease.

According to our study we found that tooth cementum annulation method is the most reliable method for age estimation when used with special microscopic methods such as phase contrast microscopy and digital image enhancement procedures.

Conclusion

Teeth are particularly useful in age estimation as they display a number of observable age related variables and they tend to remain intact under circumstances which might alter or obliterate the rest of the skeleton. Countable cemental annulations are present in human teeth, and which when appreciated can be used for estimation of age of an individual with accuracy. The histological assessment of cemental annulations using phase contrast microscope and digital enhancement narrows down the error rate to just over one year making this method more reliable. Moreover, no significant influence on annual production of incremental lines was observed in the periodontally diseased teeth. This technique may be extremely valuable in forensic medicine, forensic dentistry, and anthropology. Further studies are needed to determine the availability of TCA for individuals older than 50 years. A much larger sample size would shed more light on evaluating the impact of periodontal disease on the estimated age.

Ethical approval:

Ethical approval was obtained from the Institutional Ethical Review Board. Reference number: IERB/2015-16/16.

Source of Funding: Self

Conflict of Interest: Nil

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