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Comparison of Longitudinal and transverse sections of teeth in Assessing Cemental Annulations for Age Estimation using Bright Field and Phase Contrast Microscopy: An in-vitro pilot study

[1]Dr. Heena Zainab, MDS, Professor and Head

[2]Dr. Ameena Sultana, MDS, Senior Lecturer

[1,2]Department of Oral and Maxillofacial Pathology and Microbiology, Al Badar Dental College and Hospital, Gulbarga, Karnataka.

Correspondence Author: Dr. Heena Zainab, MDS, Professor and Head, Department of Oral Pathology and Microbiology, Al Badar Dental College and Hospital, Near PDA Engineering college, Opposite Koranti Hanuman temple, Naganhalli road, Gulbarga – 585102. Karnataka. India.

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Abstract

Background: Age estimation plays an important role in identification of an individual in the field of forensic odontology. Literature studies have revealed that tooth cemental annulations would serve as a reliable tool in establishing the age of an individual. The use of specialized microscopic methods in conjunction with longitudinal sections and transverse sections individually or in combination respectively has also being employed to enhance the assessment of the cemental annulations.

Aims: The study aims at assessment and evaluation of cemental annulations in longitudinal and transverse sections of the extracted teeth using bright field and phase contrast microscopic methods.

Materials and methods: Thirty teeth were sectioned longitudinally and thirty teeth were transverse-sectioned at mid portion of the root using diamond discs. Sections were mounted and observed under bright field microscope and phase contrast microscope. The cemental lines were assessed for age estimation by adding eruption age of that tooth to obtain the chronologic age for each individual.

Results: The estimation of age using longitudinal sections is significantly better as compared to transverse sections. There was no significant difference in estimating age using bright field microscopy and phase contrast microscopy.

Conclusion: The study concluded that, the longitudinal sections of the extracted teeth appeared to be more promising in comparison to the transverse sections using cemental annulations as parameter for estimating the age of an individual. The study did not reveal any significance difference between the two methods of microscopy. More so over, it would be advisable that larger sample size would shed more light on the obtained results.

Keywords: Forensic odontology, age estimation, cementum, incremental lines, microscope.

Introduction

Estimation of age plays a major role in identification of an individual in the field of forensic odontology.[1] Age estimation of dead bodies, skeletal remains and living individuals may help to clarify issues with significant

legal and social ramifications for individual as well as for the community.[2]

Teeth are believed to be rich sources of data on the pace of somatic development and the environment in which they are formed. Precise information on developmental rate has been inferred from incremental features in teeth, which show well-established periodicities. The counts of these incremental features can be used to map the developmental age of a tooth. The dental hard tissues are able to resist decay and degradation for a longer time as compared to other tissues of the body.[3] Recent studies show tooth cemental annulation method as a reliable tool in establishing the age of an individual. It has been hypothesized that incremental lines in tooth cementum can be used as a more reliable age marker than any other morphological or histological traits in human skeleton.[4]

Cementum is calcified tissue that surrounds the dentin. Deposition of cementum occurs in a rhythmic pattern, resulting in the appearance of dark and light bands. One pair of dark and light band represents one increment which is deposited annually. Cementum is deposited as a continuous process throughout life. Apposition of cementum on the surface of tooth roots was popularized by Gustafson as one of the factor in his method for age estimation in forensic odontology.

The cemental annulations layer is primarily composed of uncalcified dense bundles of collagen fibrils. Mineralization of these bundles is done by hydroxyapatite crystals. Change in the orientation of these bundles is responsible for the optical effect of incremental lines. This pattern is visible under the microscope as a series of alternating light and dark lines or bands.

The use of different forms of microscopy have been reported in literature. Light microscope, polarizing microscope, phase contrast and fluorescence microscopy have been used to ascertain the best method to study the cementum.

So, the purpose of this study was to compare and co-relate the efficiency of the longitudinal section and transverse section of the extracted teeth in assessment of age using cemental annulations by using two microscopic methods namely bright field and phase contrast.

Aim

To assess and compare the reliability of cemental annulations in the longitudinal and transverse section of extracted teeth using bright field and phase contrast microscopy, in estimation of age.

Objectives of The Study

- To determine the correlation between the actual age and estimated age by tooth cemental annulations method.
- To compare the reliability of tooth cemental annulations (TCA) method in longitudinal tooth sections and transverse tooth sections.
- To compare the reliability of tooth cemental annulations method using bright field microscope and phase contrast microscope.

Materials and Methods

The invitro study was carried out in the Department of Oral Pathology and Microbiology and the study sample comprised of 60 teeth, which were extracted for the purpose of orthodontic treatment. The age of the individuals (when the tooth was extracted) ranged from 11-20 years. Teeth with periapical and periodontal pathologies were not included in the study, only completely erupted teeth with root completion which are morphologically and structurally sound were included. The ground sections were made using diamond tipped disc (Figure 1) and then the sections were grinded on Arkansas stone. The sections were then cleared with xylene and mounted on glass slides using DPX mountant.

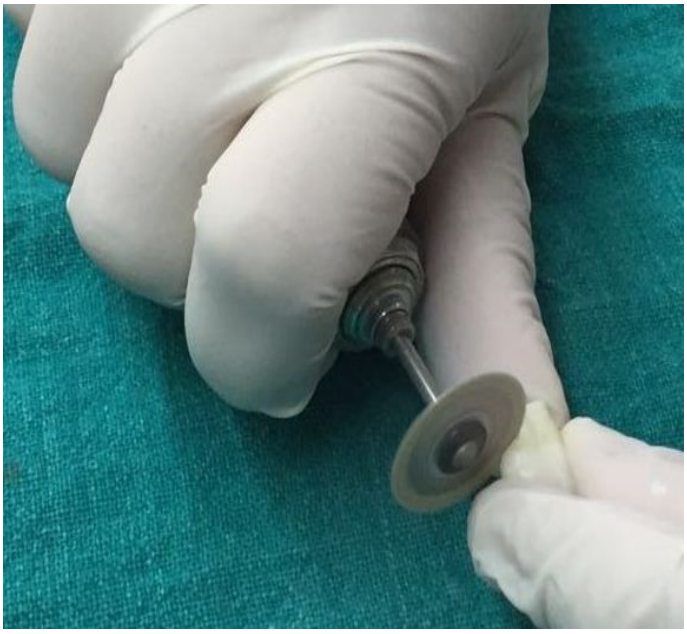


Figure 1: Original image showing sectioning of tooth using diamond tipped disc

Figure 2: Original image showing longitudinal and transverse section of tooth

Thirty teeth were sectioned longitudinally and the remaining thirty teeth were transversely sectioned at the mid portion of the root (Figure 2).

The sections were examined under bright field microscope and phase contrast microscope. In each section, the mid portion of the root was selected for counting. The mid portion of root was selected for counting the incremental lines of cementum for the following reasons:

- The width of cementum and its cellularity increases apically, which complicates the counting of annulations.
- The number of resorption areas on the root increases apically.
- The thinness of the cementum near the cervical region of the tooth inhibits scoring.
- The middle third therefore represents the best area for counting as far as layer, width, cellularity and resorption are concerned.[5]

The area selected for counting was photographed under 10x objective using a digital camera. Digital images of the incremental lines were taken from every section with a research Olympus microscope in bright field mode (Figure 3) and phase contrast mode (Figure 4). The images were magnified on the computer and the cemental lines were counted.

The eruption age of the tooth was added to the counted lines and the estimated age was obtained, as follows: Estimated age = No. of incremental lines + Eruption age of that tooth.[5]

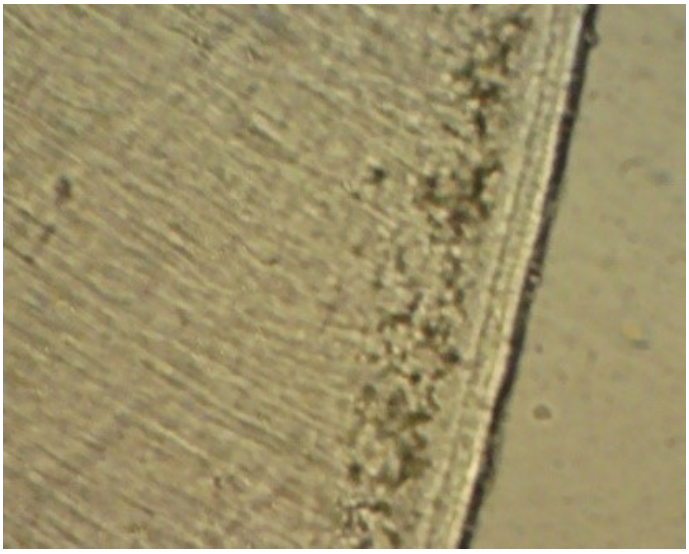


Figure: 3(a)

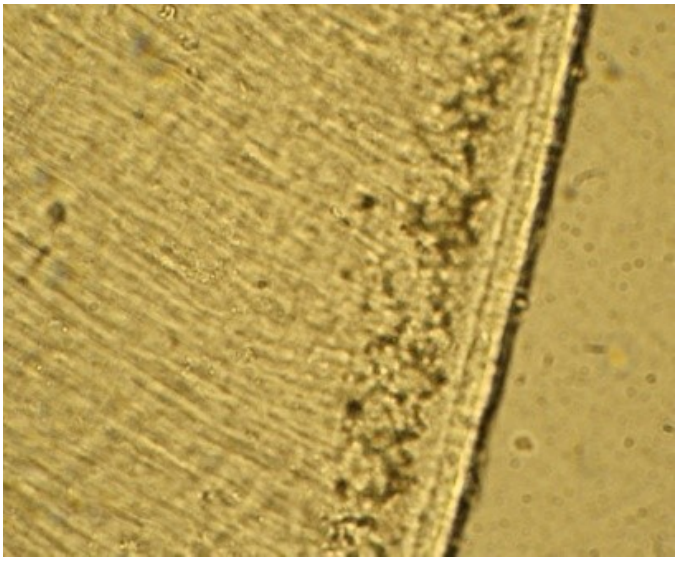


Figure: 3(b)

Figure 3: Original photomicrograph showing cemental lines in longitudinal sections under **(a)** bright field microscope and **(b)** under phase contrast microscope (Ground section, x10)

The data obtained was subjected to statistical analysis. The statistical software namely SPSS 16.0 version was used for the analysis of the data. Microsoft word and excel were used to generate graphs and tables. Student's t test has been applied to assess the significance of the study parameters. Pearson coefficient was used to assess the correlation between actual age and calculated age.

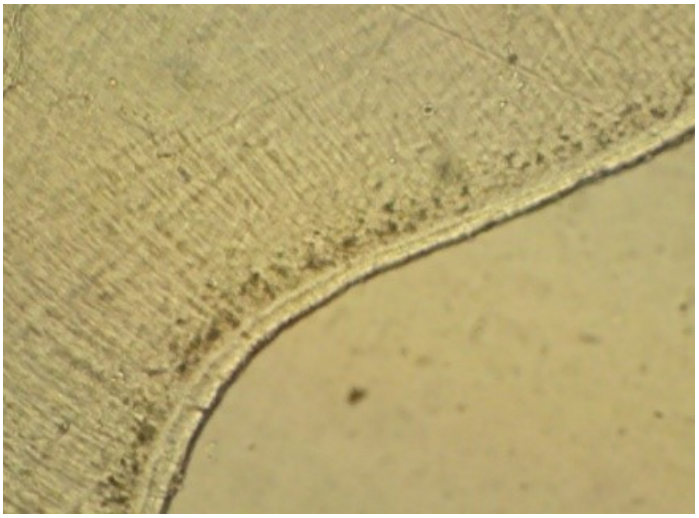


Figure: 4 (a)

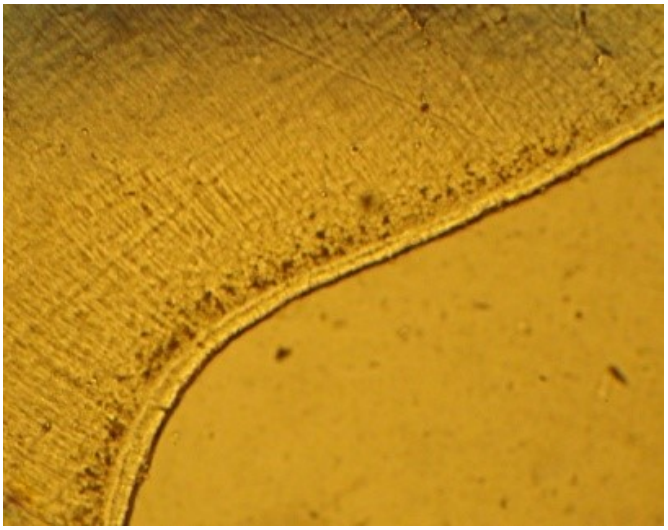


Figure: 4(b)

Figure 4: Original photomicrograph showing cemental lines in transverse sections under (a) under bright field microscope and (b) under phase contrast microscope (Ground section, x10)

Results & Observations

Sixty teeth were included in the study. Dark and light incremental bands of cementum were observed in all the specimens. Cemental annulations in longitudinal sections of thirty teeth and transverse sections of thirty teeth were counted and analyzed for their correlation with actual age of the person using bright field microscope and phase contrast microscope. The counts of cemental annulations, calculated and the actual chronological ages of individuals were tabulated for longitudinal sections and transverse sections.

Pearson correlation **(r)** of actual age and calculated age on the counts in longitudinal sections and transverse sections is represented in ***Table 1***. The Pearson correlation (r) value of transverse sections comparing actual age and calculated age is 0.906, using both bright field microscope and phase contrast microscope, which shows a positive correlation. The P value of transverse sections comparing actual age and estimated age is 0.01 for both bright field microscope and phase contrast microscope, which is

significant. The r value of longitudinal sections comparing actual age and calculated age using bright field microscope and phase contrast microscope is 0.928 and 0.910 respectively, which shows positive correlation. The P value of longitudinal sections comparing actual age and estimated age is 0.01 for both bright field microscope and phase contrast microscope, which is significant.

Table 1: **Pearson correlation of actual age and estimated or calculated age**

Pairs	Transverse section		Longitudinal section		R^2	Regression equation
	r	P	r	P		
Actual age versus calculated age using bright field microscope	0.906	0.01	0.928	0.01	0.820	Actual age=10.24+0.831 x (incremental lines)
Actual age versus calculated age using phase contrast microscope	0.906	0.01	0.910	0.01	0.846	Actual age=9.82+0.855 x (incremental lines)

The coefficient of correlation determination (R^2) is 0.820 and 0.846 for bright field microscope and phase contrast microscope respectively; which explains the variability of the age estimates around its mean.

The comparison of mean age error among transverse sections and longitudinal sections is represented in Table 2. The mean age error among transverse sections is 2.2± 0.92, that is greater than the mean age error among longitudinal sections which is 1.58± 0.63. The calculated probability (P value) is 0.018, which is statistically significant.

Table 2: Comparison of mean error among transverse section and longitudinal section

Sections	**Mean ± SD**	**t-test**	**P-value and significant**
Transverse section	2.2 ± 0.92	t = 2.44	P=0.018 Significant
Longitudinal section	1.58 ± 0.63		

Prediction of actual age using calculated age in years is also represented in ***Table 1***, which is a prediction equation by regression equation. Based on the regression equation, comparison of actual age and calculated age in bright field microscope and phase contrast microscope by longitudinal sections method is given in ***Table 3*** and by transverse section method in ***Table 4***.

Table 3**:** Comparison of Actual age with estimated age in bright field microscope method and phase contrast microscope method by LONGITUDINAL SECTIONS

Age	**Actual age**		**Estimated age in bright field microscope method**		**Estimated age in phase contrast microscope method**	
	No. of cases	**Percentage**	**No. of cases**	**Percentage**	**No. of cases**	**Percentage**
11-15	23	76.7	17	56.7	17	56.7
16-20	7	23.0	13	43.3	12	40.0
21-25	0	0.0	0	0.0	1	3.3
Total	30	100.0	30	100.0	30	100.0
χ^2 test value	$\chi^2 = 2.70$					
P-value	P>0.05					
Significance	Not significant					

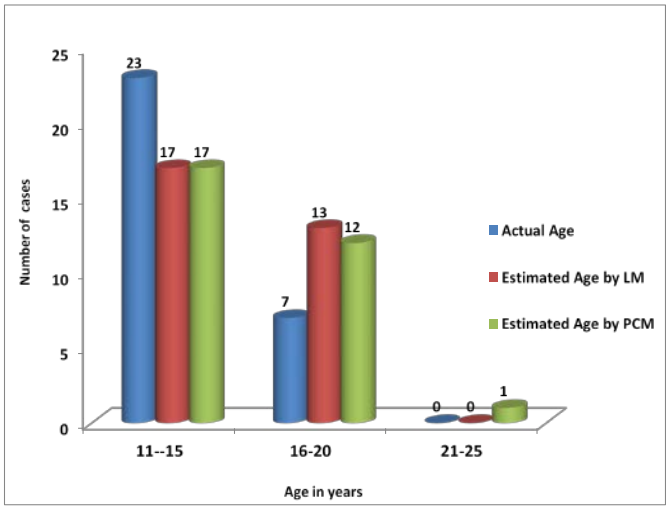
Table 4: Comparison of Actual age with estimated age in bright field microscope method and phase contrast microscope method by TRANSVERSE SECTIONS

Age	**Actual age**		**Estimated age in bright field microscope method**		**Estimated age in phase contrast microscope method**	
	No. of cases	**Percentage**	**No. of cases**	**Percentage**	**No. of cases**	**Percentage**
11-15	22	73.3	12	40.0	12	40.0
16-20	8	26.7	16	53.3	16	53.3
21-25	0	0.0	2	6.7	2	6.7
Total	30	100.0	30	100.0	30	100.0
χ^2 test value	$\chi^2 = 6.78$					
P-value	P<0.01					
Significance	Highly Significant					

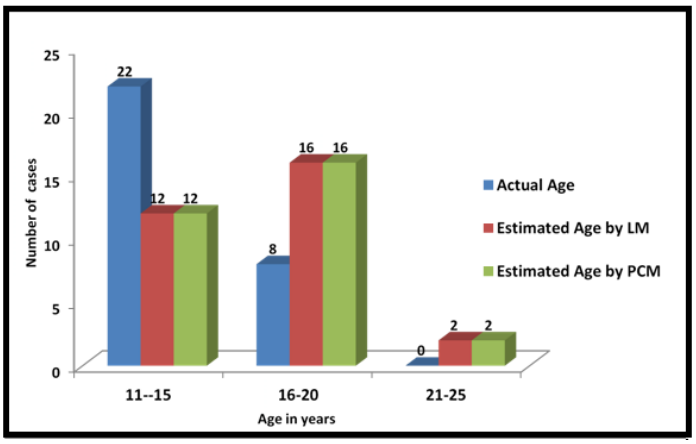
There was no statistical significant difference in actual age and estimated age in bright field microscope and phase contrast microscope by longitudinal sections, where P>0.05. There was statistical highly significant difference in the actual age with estimated age in bright field

microscope method and phase contrast microscope method by transverse sections, whereP<0.01.

Graph 1*:* Multiple bar diagram represents actual age comparison with estimated age in bright field microscope method (LM) and phase contrast microscope method (PCM) by longitudinal sections.



Graph 2:Multiple bar diagram represents actual age comparison with estimated age in bright field microscope method (LM) and phase contrast microscope method (PCM) by transverse sections.



Discussion

Cementum is a mineralized tissue covering the entire root surface. It is located in an intermediate position, at the interface between root dentin and the periodontal ligament. Although being a component of the tooth itself, functionally it is a part of the dental attachment apparatus, that is, the periodontium. Dental cementum continues to grow in thickness throughout the life.

Kvaal and co-workers have said that apposition of cementum occurs in phases, which results in two types of layers with different optical properties.[6] These incremental growth layers in human teeth are seen as narrow, dark-staining lines which alternate with wider, paler staining bands of cementum. The dark staining lines are referred to as incremental lines and the cementum between each two lines as incremental bands.

A biological explanation for these alternating layers was given by Lieberman [7] and Schroder. They suggested that the dark lines are stop phases of mineralization during continual growth of the fibroblasts, leading to a change in mineral crystal orientation.[3] This pattern appears under the microscope as a series of alternating light and dark lines or bands. These dark lines have been referred to as incremental lines of cementum.

Cementum annulations have been studied in previous literature extensively in animals. In the early 1980s, the study of human teeth proved that the Tooth Cemental Annulations (TCA) method could be applied to human teeth as it had been to other mammals previously.[7] Since then many studies have been done to prove its application in age estimation using various methods and formulae.

Scott et al, in 1982 first used TCA method, as an age estimation method in humans and found a positive correlation between human age and incremental lines.[8] Further technical improvements led to the suggestion that the TCA method is superior to other tooth-based methods of age estimation in the adult skeleton.

TCA method was not only applied to freshly extracted teeth, but was also applicable to historical skeletons and cremetions. The use of this method in forensic cases, added further support to the idea that the number of incremental lines is a stable property, even under

circumstances when other characteristics of the lines (such as the width and extent of mineralization) have been altered by environmental or physiological perturbations.[4] It was on the basis of these kind of results that the TCA method was recommended as a reliable technique for age estimation in humans using skeletal materials. This study was conducted to further assess the accuracy with which cementum annulations can be used for age estimation using bright field and phase contrast microscope.

The sectioning method of tooth has also been a topic of interest. Many authors found longitudinal sections to be appropriate, whereas others prefer transverse sections. So, in the present study we also aimed to compare the cemental annulations in longitudinal sections and transverse sections of teeth. Both sections seem to have advantages and limitations. Longitudinal sections allow examining the whole root surface such as advocated by Klevezal and Kleinenberg.[11] Stott et al.[8] (1982), Avadhani et al[5] (2009) prefer transverse sections as they allow a series of observations. Maat et al. (2006) recommended to cut the sections perpendicular to the exterior of root not perpendicular to the root axis. In this study, we found that the mean age difference was higher when transverse sections were used when compared to longitudinal sections. This indicated that longitudinal sections gave a more closer age estimate than the transverse sections.

Charles et al.[9] in 1986, systematically evaluated the distribution of lines in cementum of the middle third of root in different sections. Kagerer and Gruppe used a method called 'sequential analysis', to assess the 'most stable figure' or the 'most reliable number of layers'.[10]Wittwer-Backofen[4] and co-workers used 70−80 μm thick unstained mineralized sections for counting the lines, while Kagerer and Grupe counted lines in cementum of 70 μm thick unstained mineralized sections using phase-contrast microscopy.[10]

In our study, we found that middle third of tooth root was most suitable to count cemental annulations, similar to the studies conducted by Mallar[2] and Aggarwal.[4] The mid portion of the root has an adequate thickness and shows more parallel cemental lines which are easy to count. This region is least affected by local or systemic factors; and being acellular does not obscure the counting of lines.

The percentage of cases in the actual age group of 11-15 years was 73.3% for transverse sections whereas in the calculated age group was 40% for both bright field microscope and phase contrast microscope respectively. The percentage of cases in the actual age group of 16-20years was 26.7%, whereas in the calculated age group was 53.5% for both the microscopic methods. There were no cases in the actual age group of 21-25 years but the calculated age showed 6.7% cases using microscopic methods. The P value indicated that there was a statistically significant difference in actual age and calculated age when in transverse sections. Our study was found in accordance with the study conducted by Mallar KB, who also found a statistical significant difference with transverse sections.[2]

The percentage of cases in the actual age group of 11-15 years was 76.7% for longitudinal sections whereas in the calculated age group was 56.7% for both bright field microscope and phase contrast microscope. The percentage of cases in the actual age group of 16-20years was 23%, whereas in the calculated age group it was 43.3% for bright field microscope and 40% for phase contrast microscope. There were no cases in the actual age group of 21-25 years but the calculated age showed 3% cases using phase contrast microscope. The P value indicated that there was no statistically significant difference in actual age and calculated age when

longitudinal sections were used. This also suggested that there was no significant difference on the estimated age when comparing bright field microscope and phase contrast microscope. Our findings are again similar with the findings of Mallar KB, who reported no statistical significant difference with longitudinal sections.[2]

The use of bright and phase contrast microscopy has also been compared to judge the age using cementum annulations. Pundir et al, (2009) found that the cemental annulations were more clearly visible under phase contrast microscope as compared to polarizing microscopy and light microscopy. Tyagi et al, (2014) highlighted the reliability and importance of the age estimation criteria using cemental annulations and confirmed that the proper usage of bright-field microscopy can be effectively put to use.[14] In the present study, we found that bright-field microscopy and phase contrast microscopy showed no significant difference on counting the cemental annulations, similar to the study conducted by Prabhu and Hemavathy (2015).[15] Use of phase contrast microscopy has got an added advantage of enhancing the view for counting the cemental lines. The results obtained were same as that observed under bright-field microscope.

However, the methodology of counting cementum annulations proves useful when adequately thin sections are used. Through proper processing and with correct use of microscopic methods and photography, counts of cementum annulations would serve as a specific and reliable tool of age estimation in forensic sciences.

Conclusion

The ability to estimate the ages of victims of natural or manmade disasters would be a valuable tool in forensic dentistry and forensic medicine. The study here concludes that, countable cemental annulations are present in human teeth, and which when appreciated can be used for estimation of age of an individual with accuracy and also that tooth cementum annulation (TCA) method serves as one of the important and valuable tool for forensic identification, when used in conjunction with longitudinal sections and specialized microscopic methods.

We would finally conclude by suggesting that much larger sample size would shed more light on evaluating the best microscopic method between the bright field and phase contrast microscopy for appreciating better cemental annulations thus aiding in precise age estimation.

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