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Age Estimation in Odisha Population Using Cemental Annulation Count: A Comparison of Bright Field, Phase-contrast, and Polarized Microscopy

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***Abstract*:**

Background: Cementum is a hard avascular connective tissue that covers roots of the teeth which is deposited in layers throughout life. Microscopically each layer is seen as a set of alternating dark and light bands called cemental annulations. The objective of the present study is to examine the correlation between the number of cemental lines and age of an individual, and also to find the efficacy of bright field microscopy (BFM), phase-contrast (PH), and polarized (POL) microscopy for counting these annulations.

Materials and Methods: Extracted teeth from patients of known age devoid of any pathology were selected, and longitudinal ground sections were prepared. These ground sections were observed under BFM, POL, and PH microscopy. Microphotographs were further magnified using a computer. The dark lines on the cementum were counted using Image Pro Express 6.0 software.

Results: PH microscopy showed better correlation (0.9952) between actual and estimated ages in comparison to BFM (0.9760) and POL microscopy (0.9833).

Conclusion: Our study showed that PH microscopy can improve the precision of age estimation in forensic identification.

Key Words: Age estimation, ground section, phase-contrast microscopy, tooth cemental annulations

Introduction

Cementum is the mineralized dental tissue covering the anatomic roots of human teeth and it furnishes as a medium for attachment of collagen fibers that binds the tooth to the surrounding structures.[1]

Formation of cementum is a process which is continuous throughout life. The thickness of cementum increases by three folds between 20 and 60 years of age, which is sparse near the cement–enamel junction as compared to the apex of tooth.[2] As a general rule, however, acellular cementum is seen more frequently on the coronal half when compared to cellular cementum which is seen on the apical half of the root.

Cementum shows many incremental lines which indicate its periodic formation.[1] These incremental lines consist of a wide light layer and a narrow dark layer formed during summer and winter, respectively. The layers in cementum reflect a cyclic annual formation pattern. A pair of alternating light and dark lines corresponds to a year in an individual's life.[3]

Investigators in the field of forensics are still in quest of an accurate method for the estimation of age using body parts of the dead. Hard tissues of teeth have a better resistance to the process of degradation as compared to other tissues due to which tooth has become one of the most useful tools in the estimation of age.[4]

The total width of cementum was used first in human age estimation.[5] In 1982, Stott *et al.*[6] for the first time used tooth cemental annulation count (TCA), as a method in the estimation of age. Later, many technical modifications[7] were done and TCA was considered much superior as compared to other tooth-based methods of age estimation in an adult skeleton.

Zander and Hurzeler stated that the incremental lines present in cementum can be used as a more reliable source for estimation of age than other traits in human skeleton. This was said on the basis of biological factors of these annulations known so far.[8]

This study was done to examine the correlation between the number of cemental lines and age of the patient and also to ascertain whether bright field microscopy (BFM), polarised

(POL), or phase-contrast (PH) microscopy is a reliable method in studying cementum.

Materials and Methods

Preparation of ground sections

The study sample consisted of 353 teeth, one tooth from each individual, which were extracted due to orthodontic and prosthodontic reasons and were devoid of any pathology such as attrition, hypercementosis, or periapical pathology. Age of the study participants (at the time extraction) ranged between 20 and 70 years. All the freshly extracted teeth were preserved in formalin until ground to obtain thin longitudinal sections of 80 μm thickness using a Carborundum™ grinding stone. Broken sections and the sections which are devoid of cementum have been rejected from the study (43 ground sections). The final study sample was about 310 ground sections (one section per tooth). The sections were mounted on a slide using DPX mountant.

Microscopy and counting of cemental annulations

Each ground section was observed individually under BFM, POL, and PH microscope. For viewing annulations, area where the middle and apical third of the tooth met was chosen, and counting was done by three observers who were not aware of the actual age of the participants. A pair of light and dark lines on the cementum represents a year.[9] In our study, the dark lines counted were referred to as TCAs. Finally, an average of these TCA count by the three observers was used to evade inter-observer variability. The Wilcoxon matched test was applied to see the significant difference between two observers (Obs 1 and Obs 2) and cross-verified by Spearman's rank correlation (Table 1).

Digital images of TCA were taken from all the 310 ground sections using a binocular Olympus microscope BX51 Penta Head in BF, POL, and PH mode. Jenoptik ×3 digital camera was used to click the microphotographs under ×10 objective. All these images were magnified on Dell, Intel® Core i3, 2.00 GB RAM computer. Counting the annulations was done using the Image–Pro Express 6.0 software.

If the count of cementum annulations is added to the tooth-specific eruption age, the result is an estimate of the chronological age. This formula was used by Stott *et al.*[6] which is used in the present study.

Estimated age (E) = Number of incremental lines (n) + Eruption age of tooth (t)

Data were charted, and analysis was done using Karl–Pearson correlation coefficient.

Results

Inter-observers' reliability tested using Spearman's R value showed that observer (Obs) error has not substantially affected the results of the study (Table 1). The actual and estimated age of the patients were determined using BFM, POL, and PH microscopy. Pearson's correlation coefficient between estimated age from TCAs and actual age of the individual was found to be positive with significance at a level of 0.05 (Table 2) in the present study.

Karl–Pearson correlation coefficient was less when PH microscope was used (0.9775) in comparison with BFM and POL microscopy (Table 3). A linear regression slope was plotted where all the points clustered very close to the slope when PH microscopy was used (Figure 1) while most of them were out of the slope when BFM and POL microscopy were used (Figures 2 and 3). There was a high correlation between estimated and actual ages when PH microscopy was used (Table 4).

Discussion

Cementum is the calcified, avascular tissue which is resistant to resorption. Appositional growth occurs in cementum by

Table 1: Inter-observers' reliability.

Variables	Observers	*n*	*Z*-value	*P* value	Spearman's *r* value
LM	Obs 1 and Obs 2	10	1.6036	0.1088	0.9999*
LM	Obs 1 and Obs 3	10	0.0000	1.0000	0.9999*
LM	Obs 2 and Obs 3	10	0.8018	0.4227	0.9999*
POL	Obs 1 and Obs 2	10	0.0000	1.0000	0.9878*
POL	Obs 1 and Obs 3	10	0.0000	1.0000	0.9878*
POL	Obs 2 and Obs 3	10	1.0690	0.2851	0.9634*
PH	Obs 1 and Obs 2	10	1.0690	0.2851	0.9632*
PH	Obs 1 and Obs 3	10	0.5345	0.5930	0.9908*
PH	Obs 2 and Obs 3	10	0.0000	1.0000	0.9908*

*$P<0.05$ indicates statistically significant value, PH: Phase-contrast, POL: Polarized

Table 2: Correlation between actual age with estimated age by using different microscopes by Karl–Pearson's correlation coefficient method.

Techniques	Correlation between actual age with estimated age			
	r-value	r^2	*t*-value	*P* value
BFM	0.9760	0.9526	78.5449	0.00001*
POL	0.9833	0.9668	94.5301	0.00001*
PH	0.9952	0.9905	178.5945	0.00001*

*$P<0.05$; r is the correlation coefficient; the P value related to both t and r, whether r is significant or not is tested by t and P value. BFM: Bright field microscopy, PH: Phase-contrast, POL: Polarized

Table 3: Correlation between estimated ages using three different microscopes.

Correlation between estimated age by different forms of microscopy	*r* value (correlation coefficient)	r^2	*t*-value	*P* value
BFM×POL	0.9863	0.9728	104.8137	0.00001*
BFM×PH	0.9775	0.9555	81.1649	0.00001*
POL×PH	0.9846	0.9695	98.8201	0.00001*

*$P<0.05$ statistically significant value. BFM: Bright field microscopy, PH: Phase-contrast, POL: Polarized

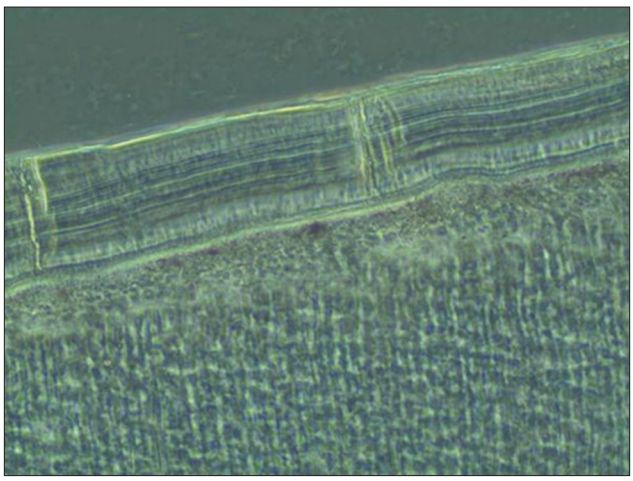

Figure 1: Photomicrograph depicting incremental lines using phase-contrast microscopy.

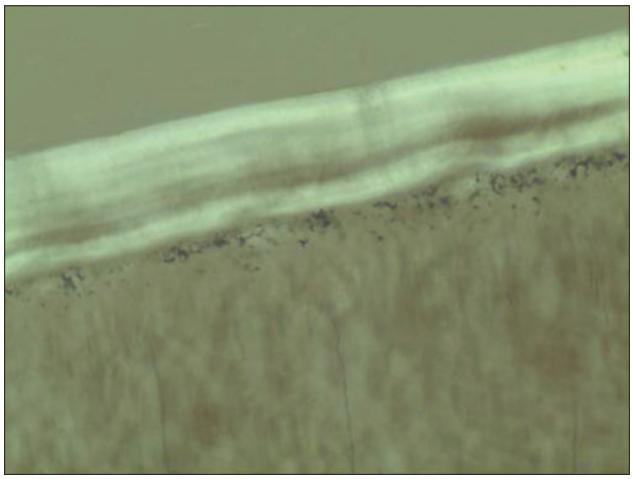

Figure 2: Photomicrograph depicting incremental lines using polarized microscopy.

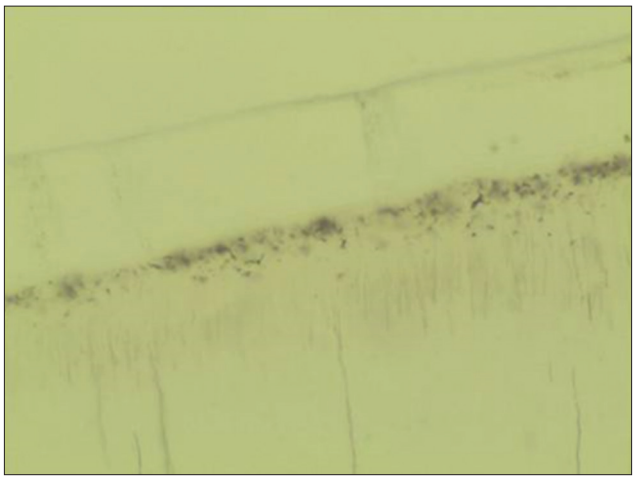

Figure 3: Photomicrograph depicting incremental lines using bright field microscopy.

deposition of increments of cementum where the thickness increases with age varying between 20 and 200 μm.[10] The process of cementogenesis starts before the tooth erupts, and the first few layers of acellular cementum are laid on the dentin. This process continues and further layers are added during and after tooth eruption.[11]

Table 4: Comparison of estimated age with actual ages by three techniques by *t*-test.

Techniques	Mean±Standard deviation		*t*-value	*P*
	Estimated age	Actual age		
BFM	40.8350±8.9404	48.4854±9.6815	−10.2051	0.00001*
POL	43.5955±9.1030	48.4854±9.6815	−6.4684	0.00001*
PH	46.8900±9.5724	48.4854±9.6815	−2.0600	0.0398*

*$P<0.05$. BFM: Bright field microscopy, PH: Phase-contrast, POL: Polarized

Lieberman[4] postulated that these dark lines are stop phases of mineralization leading to a change in the mineral crystal orientation which can be visualized under microscope as a series of alternating light and dark lines or bands. The dark lines were referred as incremental lines, and the cementum present between these two lines was known as incremental bands.

The feasibility of using cemental annulations for estimation of age in various animals[12,13] and in humans[14,15] was evaluated by many authors. The TCA method has several advantages with regard to other age estimation methods as the dental hard tissues belong to the most durable substances produced by the human body. Besides, they show the best resistance against post-mortem alterations caused by humidity, high temperature, microbial activities, and mechanical forces. Second, cementum does not undergo remodeling process as in bone. Therefore, these tissues are very useful in estimation of age.[3]

Both longitudinal and cross-sections of ground sections are preferred.[14,16] In our study, we preferred longitudinal sections as it allowed in viewing the whole root surface and we focused on the middle portion of root where acellular cementum predominates. The total sample was 353, of which 43 (12.18%) sections were discarded due to indistinct, invisible annulations.

Many factors such as nutrition, hormonal cycle, and other conditions such as temperature and ultraviolet light cause variations in the process of cementogenesis which further changes the appearance of these incremental annulations.[11] This was studied by Laws and Grue *et al.* in about 50 different mammalian species all around the world.[17,18]

Incremental lines of cementum were investigated using different microscopic techniques by Aggarwal *et al.* where they showed that the TCA was visible more clearly under POL microscope when compared to BFM.[19] In a similar study done by Kaur *et al.*,[16] cemental annulations were better viewed, and a positive correlation between the estimated and actual age was seen when polarizing microscopy was used than BFM. The visibility further enhanced when imbibing media like quinoline were used.

Kvaal *et al.*[20] found that fluorescence microscopy appears to be most useful, especially in combination with staining the sections with cresyl violet in comparison with BF, confocal, PH, POL, and interference microscopy. In contrast, a study by Pundir *et al.*[15] showed better results of TCA under PH microscopy (correlation coefficient *r* is 0.3539) than light and POL microscopy which is concomitant with our study where the correlation between the estimated age and the actual age according to the Karl–Pearson correlation coefficient was highly positive (correlation coefficient *r* is 0.9775), with significance at a level of 0.05.

Conclusion

Our research showed that counting TCAs using phase-contrast microscopy can be one of the excellent methods for age estimation in forensics where the age of the patient is unknown. This can be corroborated with studies using large samples in further advancements.

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