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Comparison of polarizing & phase contrast microscopy for estimation of age based on cemental annulations

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ABSTRACT

INTRODUCTION: Identification of age of victim by tooth remains is a very old concept .Tooth is highly mineralized structure which remains unchanged even after accidental death or death due to mass disasters. Recent research indicates that tooth cemental annulations may be used more reliably for estimation of age. **AIM & OBJECTIVES:** To examine the correlation between age & the number of incremental lines in human dental cementum using polarizing & phase contrast microscopy & compare reliability of both microscopic methods in age estimation based on cemental annulations. **MATERIALS & METHODS :** The study sample consist of 30 teeth that had been extracted from patient ranging in age from 25-26 years. Longitudinal ground section were prepared & observed under polarizing & phase contrast microscope.The cemental lines were counted on magnified images on computer. **RESULT & CONCLUSION :** There is a strong correlation between the estimated & calculative age when phase contrast microscopy was used and increased accuracy in calculating age by phase contrast microscopy than polarizing microscopy.

Key words: Forensic Odontology, Age estimation, Cemental Annulations, Phase contrast microscopy

INTRODUCTION

Identification of age of victim by tooth remains within the scope of forensic odontology is a very old concept and dates back as far as 66 A.D. at the time of Nero. An accurate method of age estimation is important for forensic investigators dealing with unknown bodies, part of bodies[1].The best method for estimating the age at death from human skeleton is currently unknown.Hard tissues of human dentition are able to resist decay & degradation long after other tissues are lost.Because of this, teeth can be a useful indicator of some past variation in diet or of metabolic diseases and can also be of use for calculation of age at time of death[2].

Cementum is the calcified tissue that surrounds the dentine and forms the attachment site for the periodontal fibers that link the tooth to the alveolar bone. Cementum is formed as a result of a continuous process throughout life and it has been shown to triple in thickness between the ages of 20 and 60 years[3].

The biological explanation for the alternating layers of cemental annulations denoted by the dark lines are the stop phases of mineralization during the continuing growth of fibroblasts, leading to change in mineral crystal orientation[2].It was hypothesized that since cemental annulations have been observed in all mammalian genera studied, they may also be

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found in humans and may be used to determine age in that genus[4]. It has also been suggested that various microscopic methods like polarizing & phase contrast microscopy have impact on the accuracy in calculation of cemental annulations. Hence the present study was conducted to compare the reliability of phase contrast & polarizing microscopic methods in calculation of age.

MATERIALS & METHODS

It is a prospective randomized cross sectional study. The study sample consisted of 30 extracted teeth that had been collected from patient ranging in age from 25 to 65 years from the department of Oral Surgery, Vasantdada Patil Dental College & Hospital , Sangli

Actual age of the patient was noted. Longitudinal ground section of each tooth was prepared using lathe & arkansas stone .Out of total 30 ground sections prepared, 5 were found to be improper for counting hence they were discarded.

Rest of the sections were viewed under polarizing & phase contrast microscope, while focusing on middle part of cementum were cemental annulations can be clearly visualized. Images were taken & magnified using computer & cemental annulations were counted .[fig 1,2,3,4]

For each alternating light & dark band of cemental annulations score of 1 was noted & total cemental annulations were counted.

Ages were calculated using formula:

Total Cemental Annulation + Eruption Age Of That Tooth & tabulated as shown in table 1

RESULT & STATISTICAL ANALYSIS

After applying Student's Unpaired 't' test, there was no significant difference between mean values of calculated age in years in Polarizing and Phase contrast methods (i.e. $p>0.05$) ; there was a significant difference between mean values of calculated age in years in Polarizing method and Actual age & Phase contrast method and Actual age (i.e. $p<0.05$) [Table 2,3,4]

From the table [2,3,4], it is noted that the calculated age in years is slightly more in Phase contrast method as compared to Polarized method. (i.e. 48.96 to 47.60) . It is also seen that the calculated age in years is less in Polarized method as compared to actual age (i.e. 50.60 to 47.60) & phase contrast method(i.e. 50.60 to 48.96)

From the table 5 it is seen that, the values of Coefficient of variation of calculated age in Polarizing method is more (i.e. 14.89%) than Phase contrast method and thus it is concluded that Phase contrast method is more reliable than Polarized method.

After applying student's 't' test there was a significant correlation between calculated age in polarized and phase contrast method (i.e. $p<0.05$) also, there is a significant correlation between calculated age by both the methods and actual age (i.e. $p<0.05$). [table 6]

DISCUSSION

The reconstruction of mortality patterns in past populations is necessary for paleodemographic analyses. The reliability of mortality reconstruction depends on individual sex and age estimates of the skeleton as a biological source of information[5]. Almost all established macroscopic methods for age estimation in the skeleton are problematic because only changes in biological age can be observed in skeletons[5,6]. High inter individual variability results in error margins that may reach 7 years, at best, for ages after skeletal growth is complete[7].

Hence ,an age-estimation method is needed that is less sensitive to continuous and non quantified age dependent changes in the skeleton. Counting the incremental lines seen in tooth-root cementum, has shown promise for age estimation[1,8,9]. Changing orientation of uncalcified dense bundles of collagen fibers in cementum may be responsible for the optical effect of alternating dark and translucent layers.Variations in cementogenesis that change the appearance of lines may be induced by different factors, including, for example,

biomechanical forces, nutrition, hormonal cycle, or ecological conditions such as temperature, ultraviolet light, humidity, altitude, or pollution[2,3].The microscopic examination of bone histology(Kerly,1970) & cementum annuli(Charles et al1986) method can be used satisfactorily to estimate age but disadvantage of requiring histological sections; although cementum annuli have been successfully used to develop wear standards ,their reliability as index of chronological age in humans is still being evaluated.[10]

In a previous studies done by Wittwer-Backofen and Buba, [2002], Charles et al. & Kagerer and Gruppe et al. the observers either had to count directly while looking through the microscope, or had to use simple photographs. Features crucial to the improvement of results include use of various microscopic methods like polarizing & phase contrast microscopy [8] & the digital image enhancement procedures[12];

In the present study, we found that the cemental annulations were more clearly visible under the phase-contrast microscope as compared to polarizing microscopy.

In previous studies, the reliability of current method of age estimations in both sexes proved to be age-dependent. With higher age, inaccuracy increased for both males and females. This led to recommendations of an age-limited applicability of this method for age estimation[11]. Hence in the present study we selected ages between 25 to 65yrs.

Impact of periodontal disease on cementum annulation & as a result on age estimation is still debatable.Second premolars,shows regular structures of the cementum band more often than any other tooth type. In these cases, exactly twice the number of expected lines are seen & can lead to false results.

Deviations of age estimation from true age may be caused by distinctive individual features such as an extremely early or late tooth eruption or disorders of the calcium metabolism.Both these reasons may lead to alterations affecting the whole dentition, which can be detectable by comparison of results obtained from different teeth of the same individual.

CONCLUSION

There exists a strong correlation between cemental annulations & chronological age of an individual. Among the methods of counting incremental lines by various types of microscopy,phase-contrast microscopy is more reliable for age estimation and may serve as a valuable aid in forensic identification.

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Fig 1: Polarizing Microscopic Image [10 X]

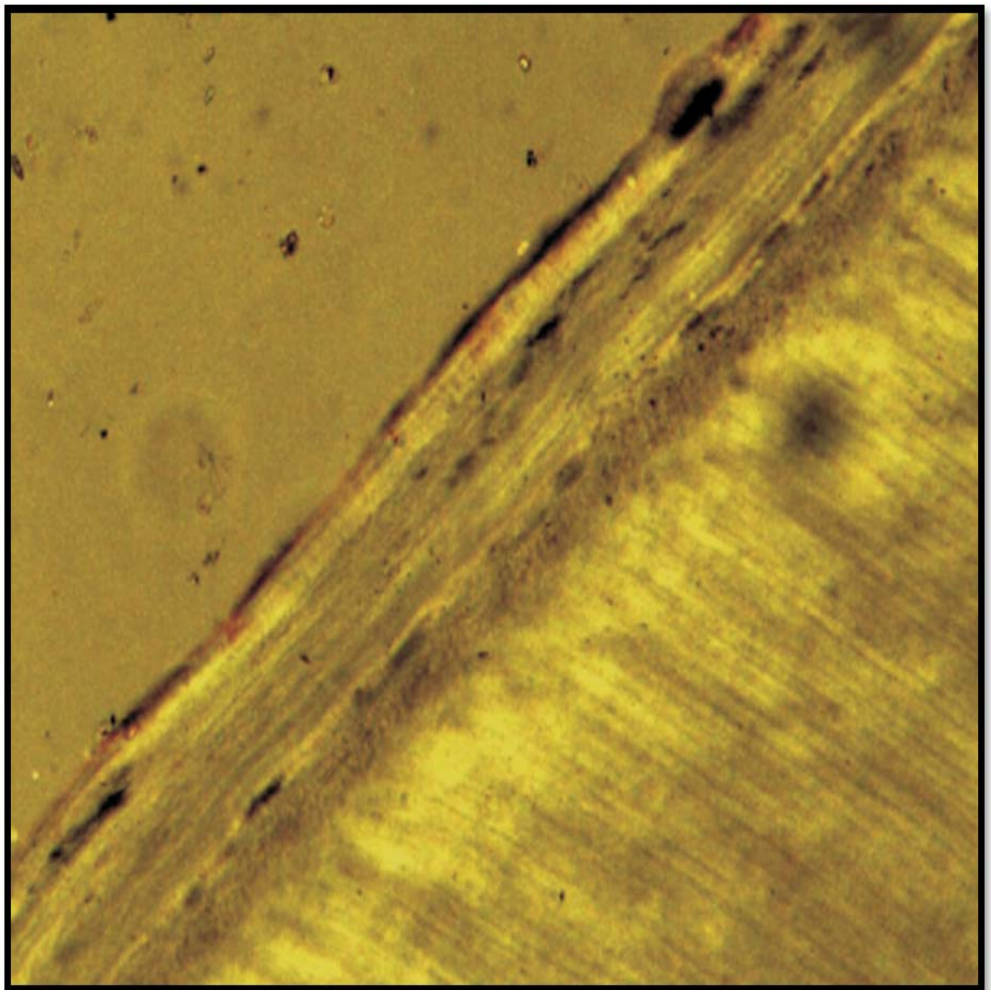

Fig 2: Magnified Image With Counting Of Cemental Lines

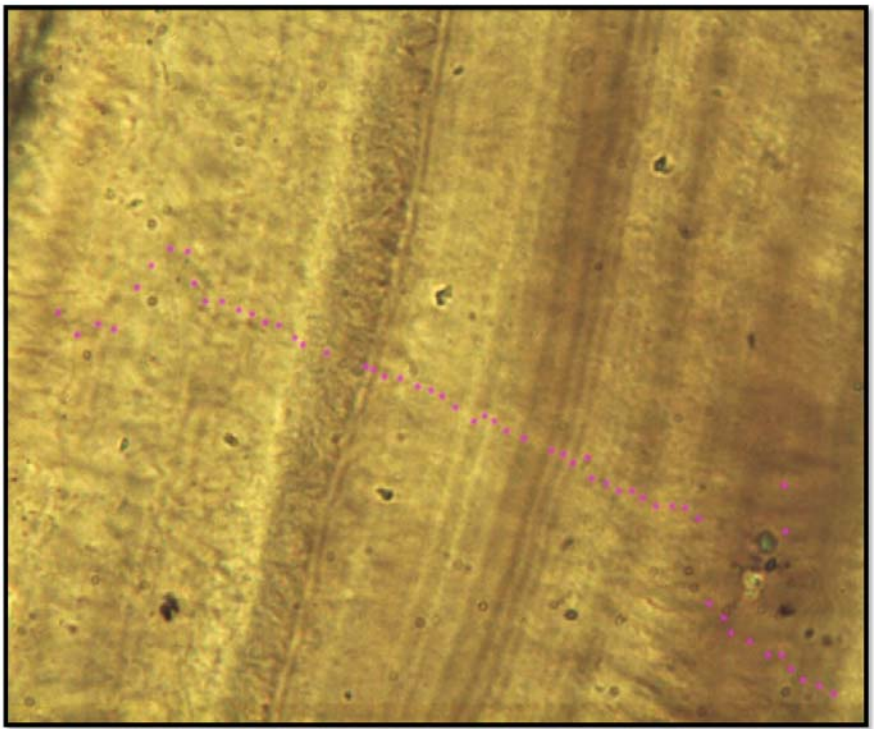

Fig 3: Phase Contrast Image [10 X]

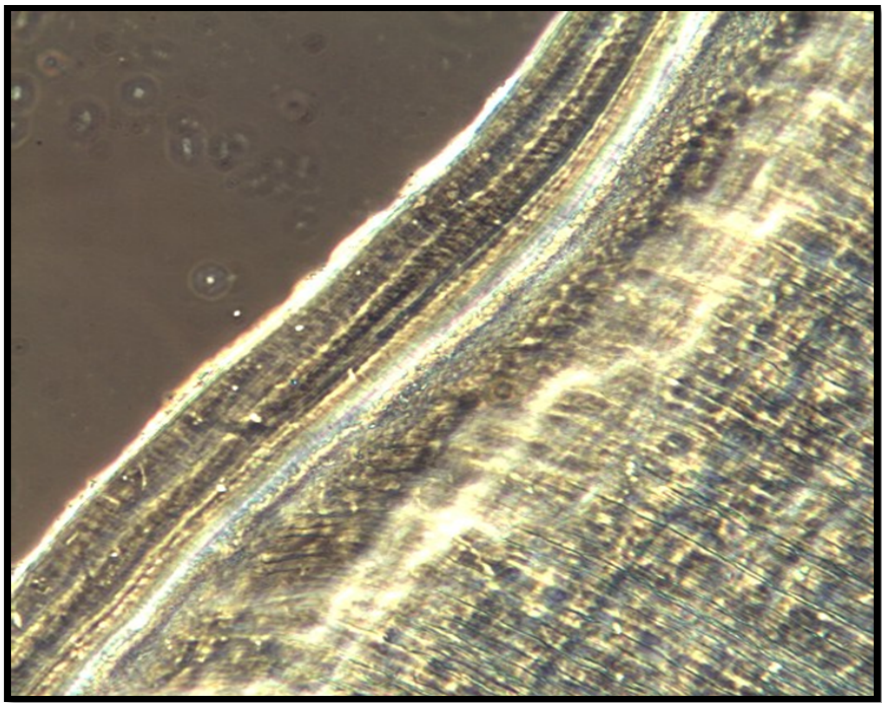

Fig 4: Magnified Image With Counting Of Cemental Lines

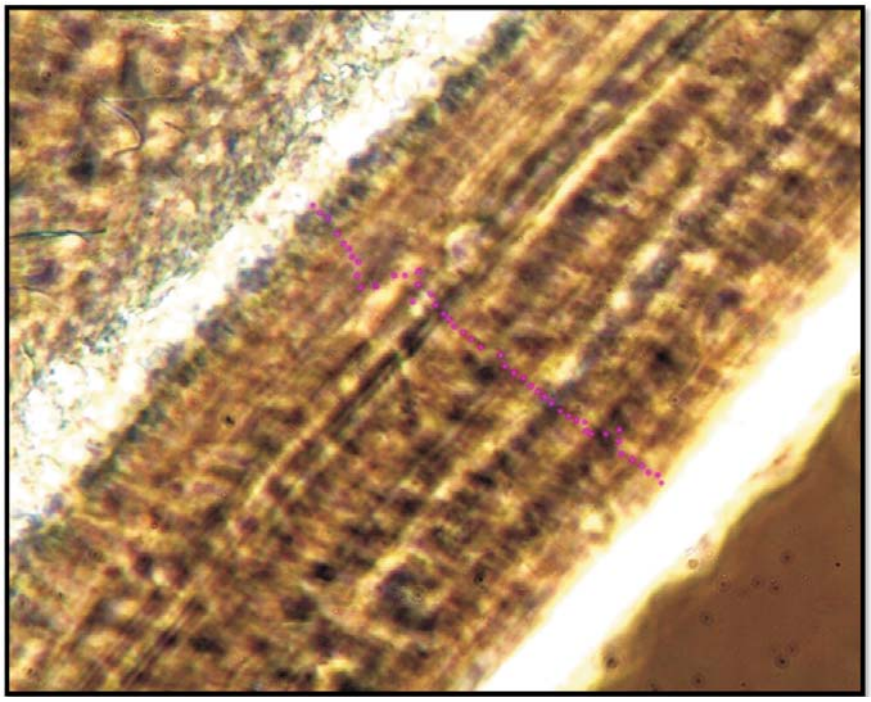

TABLE : 1

Sr. No	Tooth No.	Calculated Age[Yrs]		Actual Age [yrs]
		Polarizing	Phase contrast	
1.	12	45	46	50
2.	25	50	51	52
3.	44	53	56	55
4.	13	46	49	52
5.	44	55	47	50
6.	46	47	47	46
7.	47	35	39	40
8	22	38	53	55
9.	70	46	44	46
10.	13	45	44	46
11.	14	47	44	46
12.	17	43	51	40
13.	31	45	49	55
14.	32	36	48	55
15.	17	52	53	55
16.	16	48	57	55
17.	32	46	49	52
18.	11	48	45	55
19.	32	60	54	53
20.	15	57	56	55
21.	33	37	48	50
22.	43	59	53	55
23.	46	43	37	40
24.	47	49	52	55
25.	31	60	52	52

Table No.2: Comparison of mean and SD values of calculated age in the Polarizing and Phase contrast methods

Calculated age (years)				
Polarizing method (n=25)	Phase contrast method (n=25)	Student's Unpaired 't' test value	'p' value	Significance
Mean ± SD	Mean ± SD			
47.60 ± 7.09 (35-60)	48.96 ± 5.06 (37-57)	0.78	p>0.05	Not significant

Table No.3: Comparison of mean and SD values of calculated age in the Polarizing and Actual age in years

Calculated age in Polarizing method (n=25)	Actual age (n=25)	Student's Unpaired 't' test value	'p' value	Significance
Mean ± SD	Mean ± SD			
47.60 ± 7.09 (35-60)	50.60 ± 5.12 (40-55)	1.97	p<0.05	Significant

Table No.4: Comparison of mean and SD values of calculated age in the Phase contrast and Actual age in years

Calculated age in Phase contrast method (n=25)	Actual age (n=25)	Student's Unpaired 't' test value	'p' value	Significance
Mean ± SD	Mean ± SD			
48.96 ± 5.06 (35-60)	50.60 ± 5.12 (40-55)	1.84	p<0.05	Significant

Table No.5: Comparison of Coefficient of Variation values for Reliability of calculated age in the Polarizing , Phase contrast method and Actual age

	Calculated age in Polarizing method	Calculated age in Phase contrast method	Actual age (n=25)
Coefficient of Variation	14.89%	10.33%	10.11%

Table 6 : Karl Pearson's Correlation coefficient (r)

	Karl Pearson's Correlation Coefficient value (r)	Student's 't' test value	'p' value	Result
Calculated age in Polarized and Phase contrast method	0.5232	1.17	p<0.05	Significant
Calculated age in Polarized method & Actual age	0.3958	1.09	p<0.05	Significant
Calculated age in Phase contrast method & Actual age	0.7288	1.46	p<0.05	Significant