AN APPROACH FOR AGE ESTIMATION WITH INCREMENTAL LINES FROM ROOT CEMENTUM OF HUMAN TEETH WITH LIGHT MICROSCOPE IN NORTH INDIAN POPULATION

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ABSTRACT

Forensic age estimation plays one of the essential roles in the unknown human remains identification. Like in mass disasters and criminal investigations cases. Out of various odontological methods, analysis of incremental lines in root cementum has emerged as a non-destructive and reliable approach. Cementum, a mineralized avascular tissue covering the tooth root, continuously deposits throughout life in the form of alternating light and dark concentric bands, known as incremental lines or cemental annulations. This study explores the use of light microscopy to analyze these incremental lines for age estimation in the North Indian population. Ground sections of extracted teeth were prepared and examined under a light microscope to count the cemental annulations. The estimated age was then calculated by adding the eruption age of the respective tooth to the number of observed cemental lines. This method showed promising correlation between chronological and estimated age, supporting its validity and applicability in forensic contexts. The study reinforces the scientific rigor of cementum-based age estimation as a reliable adjunct in forensic odontology.

Keywords: Forensic odontology, Cementum, Age estimation, Incremental lines, Root cementum, Light microscopy, Ground tooth section

INTRODUCTION:

Forensic science is evolving from a long-standing reliance on "trusting the examiner" to a more rigorous emphasis on "trusting the scientific method," ensuring that forensic evidence is both accurate and legally sound.[1] The forensic odontology techniques help in the identification of the human remains in incidents such as terrorists' attacks, airplane, train and road accidents, fires, mass murders, and natural disasters such as tsunamis, earthquakes and floods, etc. (Disaster Victim Identification-DVI).

Cementum or root cementum is a mineralized tissue that covers the entire surface of the tooth root.[2,3] It was first observed microscopically by Fraenkel and Raschkow in 1835, and later by Retzius in 1836.[3] Cementum plays a crucial role in anchoring the tooth by fitting into the alveolar sockets of the alveolar bone.

Cementum is an avascular tissue as it lacks blood vessels and therefore does not undergo remodeling. However, it's thickness increases throughout life.[2]

On observing the ground sections of teeth under a light microscope, root cementum displays a alternating concentric dark and light bands, referred as incremental lines or cemental annulations. In forensic odontology, these incremental lines numbers is used to estimate an individual's age at the time of death.[4]

MATERIALS AND METHODS
The study comprised of 90 extracted teeth, collected from the Department of Oral Pathology, NIMS Dental College & Hospital, Jaipur, Rajasthan. The age of the patients from whom teeth were extracted was noted along with the reason for extraction.

INCLUSION CRITERIA
Teeth extracted from patients aged between 20-50 years for Therapeutic reasons like orthodontic or prosthodontic treatment and extraction due to periodontal diseases.

EXCLUSION CRITERIA
Hypercementosis
Endodontically treated teeth
Teeth with history of trauma
Teeth associated with cysts & tumors

Groups
Group 1: Consist of 30 individuals between age group 21-30 years.
Group 2: Consist of 30 individuals between age group 31-40 years.
Group 3: Consist of 30 individuals between age group 41-50 years.
Materials used for preparation of ground section slides:
10% neutral buffered formalin
Vaseline as separating media
Xylene
Glass slides (size 75 X 25 mm, thickness 1.35 mm)
Cover slips (size 22 X 40 mm, rectangular, thickness: 0.13 mm)
Mounting media- Dibutyl Distyrine Phthalate Xylene (DPX)

Equipments
Trinocular Olympus BX53 research microscope
Olympus SZ stereomicroscope
Computer with 17" monitor   and Intel Pentium III processor, 32bit operating system, 4 GB RAM
Operating system: Window 7 home basic.
Jenoptic scientific grade dedicated microphotographic camera
Image Analysis Software- ProgRes version 8.0 image analysis software

Preparation and Sectioning of teeth
After extraction, the teeth were rinsed in normal saline solution. They were then preserved in 10% neutral formalin until ground sections were made. The teeth were sectioned longitudinally with carborundum disc and ground section was prepared with lathe machine and Arkansas stone. The sections were dehydrated and mounted on glass slides with cover slips placed over them and observed

under light microscope.

Estimation of age using cemental annulations

In each ground section, the area at middle third of root, an area where the lines seen to be run approximately parallel was selected & photographed using Microphotographic camera.

The width of the cementum (X) from Dentino-Cementum Junction to the surface of the cementum was measured with the help of Image Analysis Software- ProgRes version 8.0. Measurement of width occupied by the two adjacent incremental lines (Y) was made. Then the number of incremental lines (N) in the total cementum width was calculated using the formula: $N = X/Y$

The age of the individual was estimated by using the formula obtained from linear regression analysis. Age $=17.384+0.786*(x)$. Where x is the number of cemental annulations.

RESULTS

The readings were subject to statistical analysis. Mean of chronologic age and mean of estimated age by using the formula obtained from linear regression analysis was calculated. The difference between mean chronological age and estimated age was calculated and a group wise difference between the mean chronological age and estimated age was calculated. The data obtained were compared using one-way ANOVA and Tukey's Post Hoc test. The mean number of cemental annulations is increasing significantly in all the age groups with increase in actual age. Intragroup comparison also has shown significant differences in between all the age groups. (Table 1).

Table 1

Cemental annulations group	Mean ±SD	P-value	Post hoc tukey test (intra group comparison)
C1	26.9±2.65		**CI vs C2**-0.001*
C2	33.95±6.81	0.001*	**C1 vs C3**-0.001*
C3	45.3±2.5		**C2 vs C3**-0.001*

*. Correlation is significant at the 0.05 level. Table 1: Inter group and intra group comparison of number of cemental annulations

The mean value of estimated age using cemental annulations showed a gradual increase in the number of annulation lines with chronological age and the difference is significant. The mean age in the C1 was 26.9±2.65 years and in C2 it was 33.95±6.81 while the third group C3 has shown a mean age of 45.3±2.5 years. C1, C2 & C3 have shown estimated mean age closer to the chronologic mean age. The estimated age using cemental annulations showed a significant difference of 7.05 years between C1 & C2, C2 & C3 has shown a difference of 11.35 years in between the groups which is significant.

When the chronological age and estimated age of all the groups were compared, the mean age difference between estimated age and actual age using cemental annulations (p=0.196) have shown no significant difference between the estimated age and chronological age. (Table 2)

Table 2: Comparison of chronologic age and estimated age for cemental annulations of all groups combined.

	Chronological age		Estimated age		P-value
	Mean	**SD**	**Mean**	**SD**	
Cemental annulations	35.98	8.02	35.55	7.47	0.196(NS)

DISCUSSION

Forensic dentistry is related to proper handling & examination of dental evidence. It deals with recognition based on features consist of an individual's dental structures. Among this, estimation of age

has a major character in the forensic identification.[1] Age estimation is a subtle discipline of the forensic sciences and it should be a necessary part of each and every identification process, especially when information which is related to the deceased is unavailable. When individuals have experienced changes so extensive that external features no longer provide useful information, teeth often remain the only reliable means of identification. The hard tissues of the human dentition are highly resistant to decay and degradation, persisting long after other bodily tissues have deteriorated. This resistance has made teeth most important indicators for evaluating variations in diet, expression of metabolic diseases, and estimation of age at the time of death.[2] The main age estimation tool are the slow changes in structure of

teeth occurring throughout life. The enamel, dentin, and cementum that constitute teeth are used to calculate the chronologic ages of unknown individuals.[3] Hard dental tissues (cementum, dentine and enamel) are hard and could be well preserved in the soil, while the enamel itself has a feature that remains unchanged over period of time and it is resistant to taphonomic changes. Many studies have developed techniques to evaluate age-at-death for adults by using the dentition tissues and dental morphology. Although the structure is not well known, in almost majority of mammalian species the numbers of bands in the dental cementum are correlated with the age at death (Gordon 1993). Studies of incremental structures in the dental cementum can be traced back to Malpighi's study of cementum in the 1600's (cited in Gordon 1993).[4]

Cementum is a calcified tissue that surrounds the root portion of dentin and serves as the attachment site for periodontal fibers, which anchor the tooth to the alveolar bone. During cementum formation, an alteration is observed between a hypermineralized layers of extracellular matrix and a less-mineralized layers. A biological explanation for these stands in layers was given by Lieberman (1994)1 and Schroder (2000).[5] The dark lines are recommended as the end phases of mineralization during continual fibroblasts growth, thus resulting in difference in mineral crystal orientation. This arrangement is visible under the microscope as a series of alternating light and dark lines or bands. The dark lines have been called as incremental lines and the cementum between each two lines as incremental bands.[6]

In a study done by Anil patel et al (2022) considering 30 freshly extracted single rooted teeth except permanent maxillary 1st premolar with age range 21-70 years. Since they believed multiple rooted teeth might cause problems during sectioning of tooth in mesio-distal plane, as shown by Backofen UW (2004) et al, that multi-rooted teeth show irregular structures of cementum band more often. and the small, narrow roots are difficult to section in the mesio-distal plane.720 to 60 years of age was considered as it's not the developing stage of tooth, and cementum triples in thickness between this age group, which coincided with Pundir et al. (2009)[8] At the beginning, the TCA technique was used on freshly extracted teeth, but Grosskopf (1989)7 showed that the method was also useful for historical skeletons and cremations. This was firmly established by others and extended to forensic cases.

The result in our study was similar to study carried out by and Pundir et al (2009)[8]. Charan Gowda et al. (2014)[10] carried out study for counting in incremental line of cementum in phase contrast microscope. Joshi et al. (2010)[11] carried out study for counting in incremental line of cementum in phase contrast microscope and polarized microscope. A result showed that less number of incremental lines of cementum was seen in light microscope followed by polarized microscope This was similar to study carried out by Pundir et al (2009)[8], Joshi et al. (2010)[11], Tupkari et al (2009)[12], Kaur et al (2009)[13], Marija et al (2012)[14], Naik et al (2014)[1], Priya et al (2014)[15], Anil et al(2022)[16]

In present study we counted range and mean value of incremental lines of cementum and the results suggest that range and mean values of incremental lines of cementum increased with increase in age. This was similar to study carried out by Pundir et al (2009)[8] and Joshi et al. (2010).[10] Incremental lines of cementum increase as age increases because cementogenesis is continuous throughout the life. But With higher age (61 - 70 yrs), inaccuracy increased but in our study we took 50 years as our upper limit. This induce supports of an age-limited applicability of this method for age estimation. Solheim T, (1992), also showed that cementum apposition decreased by one-third after the age of 60 years so that annulations for individuals above 60 years of age showed an increased tendency to be unrepresentative.

CONCLUSION

Incremental lines of cementum increases, with increase in age. From the results, we proved that the visibility and countability of lines in sections from the middle third of the root. Cemental annulations are present in humans, which is suggestive of age. Any tooth or teeth series can be used till the cementum is unharm. Thus, from microscope view, it is confirmed that our study are considered as one of the reliable age estimation method based on incremental lines of cementum. The study should be conducted further for a detailed investigation on the impact of different individual parameters such as sex, tooth position, and presence of periodontal disease.

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