Original Study

AGE REVEALING ANNULATIONS: APPLICATION FOR ESTIMATION OF AGE IN CENTRAL INDIA POPULATION

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ABSTRACT

Objective: - The purpose of this study was to examine the correlation between age and the number of incremental lines in human dental cementum and to ascertain which, among three different forms of microscopy.

Background: - Age estimation is an important factor in the identification of an individual in forensic science.The hard tissues of the human dentition are able to resist decay and degradation long after other tissues are lost. This resistance has made teeth useful indicators for calculation of age at death of an individual. Recent research indicates that tooth cementum annulations (TCA) may be used more reliably than any other morphological or histological traits of the adult skeleton for age estimation.

Materials and Methods: - Fifty nonrestorable teeth were extracted from patients ranging in age from 20-70 years of central india population, and longitudinal ground sections of each tooth were prepared and examined under light microscopy, polarized microscopy and phase-contrast microscopy. The cementum was composed of multiple light and dark bands that were counted on the photomicrograph with the help of Image Analysis Pro 6.0 software and added to the average eruption time of individual tooth. The predicted age of the individual was thus obtained.

Result: - It showed a significant correlation between the predicted age and actual chronological age of the individual when phase-contrast microscopy was used; the correlation was less for light and polarized microscopy. These data indicate that quantitation of cementum annulation is a moderately reliable means for age estimation in humans.

Conclusion: - We conclude that among the methods of counting incremental lines by various types of microscopy, phase-contrast microscopy improves the accuracy of age estimation and may serve as a valuable aid in forensic identification.

Key Words: - Age Eestimation, Cementum Annulations, Microscopy.

Introduction

Forensic odontology or forensic dentistry, as defined by Keiser–Neilson in 1970, is 'that branch of forensic medicine, which in the interest of justice deals with the proper handling and examination of dental evidence, with proper evaluation and presentation of the dental findings.'[1] An accurate method of age estimation is important for forensic investigators dealing with unknown bodies, parts of bodies, or skeletons. The best method is unknown for estimating the age at death from human skeletal tissue.[2]

Teeth can persist long after other skeletal structures have succumbed to organic decay or destruction by elements, such as fire. Being the most indestructible part of the body and exhibiting the least turnover of natural structure, they not only survive death, but also remain relatively unchanged for many years.[1]

Cementum is a calcified tissue that surrounds the dentine and forms the attachment site for the periodontal fibers that link the tooth to the alveolar bone.[3] Cementum is formed as a result of a continuous process throughout life and it has been observed to triple in thickness between the ages of 20 and 60 years. The thickness varies, being maximum at the apex and minimum near the cementoenamel junction.[4] Cementum layers consist primarily of uncalcified dense bundles of collagen fibrils. These bundles later become mineralized by hydroxyapatite crystals, whose changing orientations may be responsible for the optical effect of alternating dark and translucent layers.[3] Dark lines are the stop phases of mineralization during the continuing growth of fibroblasts, which leads to change in mineral crystal orientation. This pattern is visible under the microscope as a series of alternating light and dark lines or bands, known as incremental lines of cementum.[2,5] Recent research indicates that tooth cementum annulations (TCA) may be more reliable than any other morphological or histological traits of the adult skeleton for age estimation.[6]

The purpose of this study was to examine the correlation between age and number of incremental lines of cementum and to verify whether light microscopy, polarized microscopy, or phase-contrast microscopy were reliable method for studying human dental cementum.

Materials and Methods

Preparation of sections

The present study was carried out in the Department of Oral Pathology and Microbiology, Swargiya Dadasaheb Kalmegh Smruti Dental college and Hospital, Nagpur. The study sample comprised of 50 extracted teeth of central india population. Extracted teeth used in the study were those obtained from periodontal disease, impacted, caries, or orthodontic and prosthetic reasons. Teeth with periapical pathologies were not included in the study. The details of samples included in study are presented in Table 1.

Teeth included in the study Group	Teeth Number
Maxillary central incisor	3
Maxillary Canine	2
Maxillary 1st Premolar	8
Maxillary 2nd Premolar	5
Maxillary 3rd Molar	6
Mandibular Central Incisor	3
Mandibular Canine	7
Mandibular 1st Premolar	6
Mandibular 2nd Premolar	2
Mandibular 3rd Molar	8

Table 1: - Type and Number of teeth used in the study.

10% buffered formalin was used to store the extracted teeth. Care was taken to ensure the integrity of root cementum after extraction of the teeth. The age of individuals was noted at the time of extraction which ranged from 20–70 years. Consent was signed from the concerned individual for each tooth used in this study. Ground sections of the teeth were prepared. Pumice slurry was applied to the teeth surface and a polishing brush was used in a slowly rotating handpiece to clean the teeth. The teeth were then thoroughly washed using running tap water. Each tooth was then cut into sections using a diamond tipped disc, sectioning was done almost perpendicular to the long axis of the tooth. The sections were again rinsed under running tap water to get rid off debris and particles. The teeth were then ground on Arkansas stone with water to 80 µm thickness. The sections were then dehydrated, cleared with xylene, and mounted on glass slides.

Microscopy and line counting

Longitudinal ground sections of each tooth was prepared as shown in Figure-1 and examined under light microscope, polarized microscope, and phase-contrast microscope. In each section, the area at the junction of apical and middle third of root and the area where lines were easiest to count, irrespective of whether the cementum was cellular or acellular, was selected for counting.

Figure 1: - Ground sections mounted on slide.

Digital images of the incremental lines were taken from every section with a binocular Olympus microscope (CX-31) in bright field mode, polarizer mode, and phase-contrast mode. Micrographs were taken with a 10x objective with the help of Nikon 7.1x wide optical zoom ED VR camera. (Figure 2-4) The images were magnified on the computer and the cemental lines were counted with the help of Image Analysis Pro 6.0 software. According to few authors, a pair of light and dark lines represents 1 year. In the present study, we counted only the dark lines, the line count mentioned in this study, therefore, always refers to the number of dark lines. The number of incremental lines were counted in bright field microscope, polarized microscope, and phase-contrast microscope.

The lines were counted three times, separately, by three different observers to avoid any intra-observer error.

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 (n) + Eruption age of tooth (t).

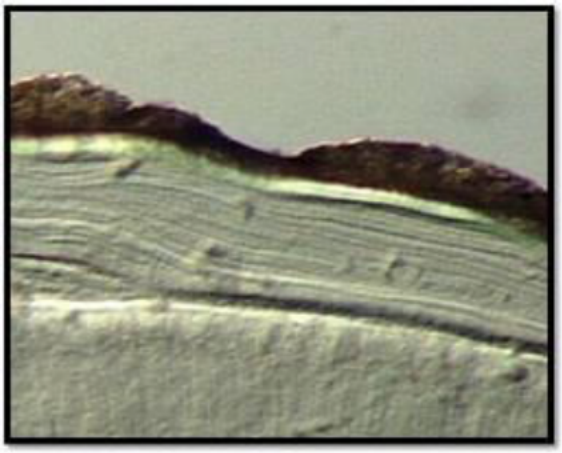


Figure 2: - Cemental annulations seen under Light microscopy

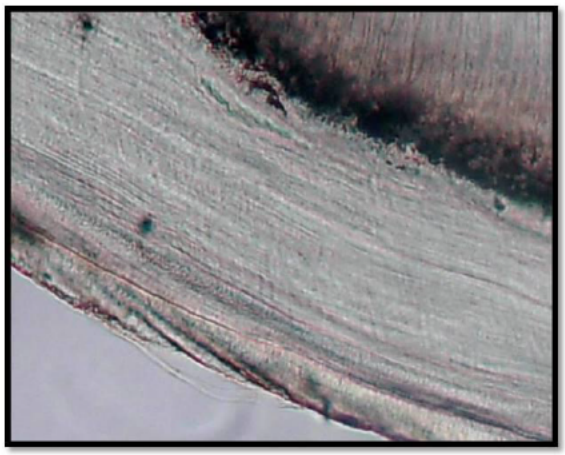
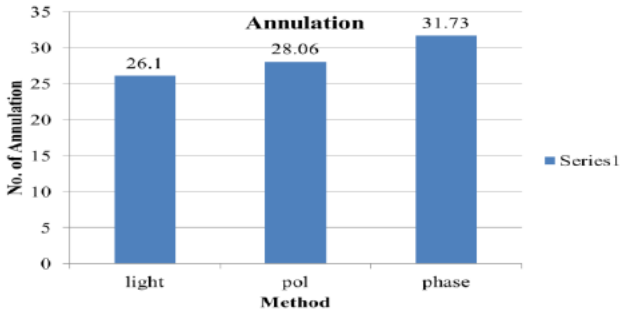


Figure 3: - Cemental annulations seen under Phase-contrast microscopy

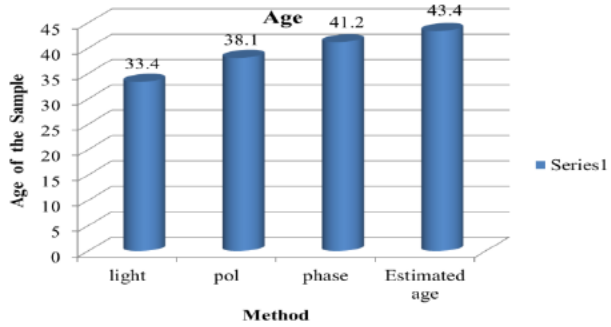
Figure 4: - Cemental annulations seen under Polarized microscopy

Results

The data obtained were analyzed using Student's t test. Some of the sections were damaged during sectioning or grinding, completely or some parts of cementum was broken, such cases were rejected. Only intact tooth sections were included in the study. About 65 teeth were initially taken for the study, out of which 15 were discarded. Only 50 sections of 50 teeth (one section per tooth) were included in the final sample. The actual and estimated ages of the persons based on all the three methods—Light microscopy, Polarized microscopy, and Phase-contrast microscopy were noted. Data was tabulated and the result is as seen in Graph 1 and 2.



Graph 1: - Number of annulations calculated by 3 different microscopic methods



Graph 2: - Distribution of age estimation by different microscopic methods in comparison with the estimated age of the patients.

Discussion

Cementum is the calcified tissue that surrounds the root portion of dentin and forms the attachment site for the periodontal fibers that links the tooth to the alveolar bone. In cementum formation, hypermineralized layers of extracellular matrix alternate with less-mineralized layers. The first layer of acellular cementum is produced before the tooth erupts, and further layers are added during and after eruption.[3]

The feasibility of using cemental annulations in human root cementum for age determination have been evaluated in previous studies. Charles and co-workers[7] in 1986, systematically evaluated the distribution of lines in cementum of the middle third of root in different sections. Kagerer and Gruppe[8] applied a method called 'sequential analysis', where they tried to find the 'most stable figure' or the 'most reliable number of layers'. Wittwer-Backofen and co-workers[3] used 70–80 μm thick unstained mineralized sections in which storage time was not reported for counting the lines, while Kagerer and Grupe[8] counted lines in cementum of 70 μm thick unstained mineralized sections using phase-contrast microscopy.

In the present study, we found that the cemental annulations were more clearly visible under the phase-contrast microscope as compared to polarized microscopy and light microscopy. Along the axis of tooth root there are two zones of different cementum types: the acellular cementum, which mainly covers the cervical part of the root, and the cellular cementum, which covers the apical part of tooth root. In the present study, root showing both cellular and acellular cementum was focused. Cemental annulations appearance, which has been observed in more than 50 different mammalian species all over the world, is said to reflect the natural metabolic rhythm of seasonal changes.[9-11] Kvaal and co-workers[12,13] have said that apposition of cementum occurs in phases, resulting in two types of layers with different optical properties. In their study, cementum was studied using conventional light microscopy, polarized microscopy, and phase-contrast microscopy. The distance from one cemental line to the next represents a yearly incremental deposition of cementum in many mammals, and counting of these lines has been used routinely for estimation of age for animals. Incremental lines in cementum observed in sections of human teeth can be prepared and stained by many methods for counting.

There are several techniques available for staining of Longitudinal and transverse sections, which can be cut from formalin-fixed human dental roots either paraffin-embedded or frozen sections. The other methods available are fluorescence, confocal laser scanning, interference contrast, and scanning electron microscopy.[12] Incremental lines in cementum can be observed in decalcified sections by using toluidine blue, cresyl violet, hematoxylin, or periodic acid-Schiff stains by conventional light microscopy, but the results are not satisfactory. Since incremental lines are not destroyed by acids and stain differently than the remaining cementum, it is likely that

they possess an organic structure which differs from that of the cementum.[12,13]

In 1967, Klevezal and Kleinenberg[14] found that the number of incremental lines counted on a sectioned tooth equalled the age in years. The robustness of the incremental lines varies among species. Especially in smaller species, the lines may be difficult to count. Phillips and co-workers[15] in 1982 examined incremental lines in two species of bats whose age was known (Myotis lucifugus and M. velifer). They found that the number of incremental lines observed depended on the tooth that was extracted and on the sections examined, and suggested that several factors, such as mechanical stress and dental drift, can affect the temporal patterns of appositional growth, resulting in non-annual cycles of dentin and cementum deposition.

In this study, we confirmed the visibility and countability of lines in sections from the middle third of the root. In humans, cemental annulations are present and can be indicative of age. As long as the cementum is intact, any tooth or series of teeth can be used.

Conclusion

Countable cemental annulations are seen to be present in human teeth. Annulations counted from a photograph or an image analyzer provides a close estimate of the actual age of the individual from whom the tooth has been extracted.

The use of this method of counting cemental lines improves the accuracy of age estimation and also makes age estimation possible in cases where only poorly preserved skeletal fragments are available. If the chronological age of the oldest individual in a historical population can be accurately determined by this method this will allow us to estimate life expectancy and the distribution of life spans within the population under study in a better way.

This study demonstrates that incremental lines are best viewed while using phase-contrast microscopy, as compared to polarized and bright field microscopy, and this method may be served as a valuable aid for forensic identification.

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