

Cementum annulations and age determination

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Over the past 30 years, scientists have used cemental annulations to reliably determine age of various animals such as otter,^[1] caribou,^[2] moose,^[3] squirrel,^[4] bear,^[5] and bat.^[6] Some investigators believe that cemental annulations are due to seasonal metabolic changes or nutritional variations due to hibernation; however, countable annulations have been found in non-hibernating animals as well.^[7]

In humans, positive association between cemental annulations and age determination has been reported by Stott *et al.*^[8] However, Miller *et al.*^[9] and Lipsinic *et al.*^[10] have reported that cemental annulations cannot be used as reliable age criteria.

Due to these conflicting reports, the present study was carried out in an attempt to throw more light on cemental

annulations and its correlation with chronologic age.

Materials and Methods

Extracted teeth from patients of known age were obtained from the Department of Oral and Maxillofacial Surgery, Government Dental College and Hospital, Mumbai. The teeth included in the study were functional and devoid of any pathology like attrition, hypercementosis, etc. Twenty-five teeth were selected. They were preserved in formalin overnight and then washed in running water before they were ground to obtain thin sections. Sectioning was done using a carborundum™ grinding stone. Half of the teeth were sectioned longitudinally and the remainder were cross-sectioned. The sections were mounted on a slide using a DPX mountant. These sections were visualized under a bright-light microscope.

Abstract

Background: Cementum is a hard tissue in the root, which is deposited around dentin in layers throughout life. Under the light microscope, root cementum is seen as alternate light and dark rings known as incremental lines of cementum. In paleontology and forensic medicine, the number of these incremental lines is used to derive the age at death of an individual. **Objectives:** The present study was undertaken to determine if any relation exists between incremental lines of cementum and age of the individual. **Materials and Methods:** Twenty-five teeth from patients of known ages and devoid of any pathology such as attrition or hypercementosis were selected for this study. Ground sections were prepared manually. Nineteen of them showed visible countable annulations, while six showed indistinct, invisible annulations and hence were excluded from the study. Half of the selected teeth were sectioned longitudinally and the remainder were cross-sectioned. The mid-root region was selected for counting the annulations. Cemental annulations were counted after taking a photograph and enlarging the mid-root area. Age was then determined by adding the eruption age of the tooth to the annulations counted. **Results and Conclusions:** It was observed that cemental annulations, when appreciated, can be used as a reliable guide to determine the age of the patient. The age thus determined varied by about 2–3 years from the actual age of the patient. The reliability of the method was found to be 94.73%. There was good interobserver agreement in counting annulations.

Key words: Age determination, cemental annulations, eruption age

Only those teeth with cemental lines which were suitable for counting were selected. Each dark band along with the light band following it constituted one annulation. Teeth with indistinct, invisible cemental lines were eliminated. The mid-root section was chosen for counting the annulations for the following reasons: 1) The thickness, width, and cellularity of the layers of cementum increases apically, thereby complicating the counting of annulations; 2) the number of resorption areas also increases apically; 3) the thinness of the cementum near the neck of the tooth inhibits scoring; and 4) to minimize the influence of factors known to obscure annulations or produce variation in cementum, such as periodontal disease and hypercementosis due to local or systemic disease. The middle third therefore represents the best compromise as far as layer width, cellularity, and resorption are concerned. The area selected for counting was photographed under a 10× objective of light microscope using a Nikon L5 camera and digitally zoomed 5 times. Counting was done on the enlarged photograph [Figure 1]. The counted cemental lines for each tooth were added to the eruption age of that tooth to obtain the chronologic age for each patient. Data was analyzed by computing the linear regression slope. We also tested interobserver agreement. A non-dental professional was chosen. This was done to eliminate bias. The criteria and method of counting were explained to him and he was asked to count the annulations in the same cemental area. Kappa statistics were used to compare our readings with those obtained by the non-dental professional.

Results

Twenty-five teeth were selected for this study. Concentric dark and light cemental bands were not clearly observed in all the specimens. Six (24%) specimens demonstrated obscured, indistinct, or invisible annulations [Figure 2]. Interestingly, five (83%) of these were in longitudinal sections and only one was in a transverse section. These teeth were deemed inadequate for counting and were eliminated from the study. Only 19 specimens with visible countable annulations were thus used in this study [Figure 3]. Transverse sections were found to be better than longitudinal sections for the purpose of counting cemental lines.

The total number of counts for each specimen was added to the eruption age of that tooth to get the estimated age of that patient. Comparison between the estimated age and chronological age was done by plotting a linear regression slope [Figure 4] and a bar graph [Figure 5]. It was found that all the points were clustered very close to the slope, except in one case. Four points were on the slope. This indicates the high reliability of this method. In 18 of the 19 specimens, the estimated age varied from the chronological age by about 2–3 years. The reliability of the method was 94.73%.

The kappa value for interobserver agreement was 0.7. This indicates that there is substantial agreement between the observers in counting the lines.

Discussion

Many researchers have suggested the use of cementum of teeth for determination of human chronologic age. The composition of cementum is similar to that of bone: 60% mineral and 40% collagen. Cementum is avascularized and resistant to resorption. Appositional growth occurs in cementum by deposition of increments of cementum. Its thickness increases with age and varies between 20–200 μm .^[11]

Cemental annulations have been reliably used for age determination of various animals, including otter,^[1] caribou,^[2] moose,^[3] squirrel,^[4] bear,^[5] and bat.^[6] Some investigators believe that cemental annulations are due to the result of seasonal metabolic changes or nutritional variations due to hibernation; however, countable annulations have been found in non-hibernating animals as well.^[7] In humans too cemental annulations are present and can be used to determine age. In this study, we selected the mid-root area of a sample of teeth for counting annulations. Microscopic examination revealed that 24% of the specimens had obscure, indistinct, invisible cemental annulations that were not suitable for counting. Lipsinic *et al.*^[10] and Miller *et al.*^[9] have reported similar findings, concluding that cemental annulations failed to predict chronologic age. The remaining 76% of specimens in the present study demonstrated countable annulations. The age estimated from these specimens was within 2–3 years of the actual chronological age of that patient, thus showing that they are fairly reliable guide for age determination. These findings matched those published by Stott *et al.*^[8] who carried out a study on teeth extracted from cadavers. When computer software was used for counting the annulations by Wittwer-Backofen *et al.*,^[12] the variation between the actual and estimated age was found to be in the range of 2–3 years. Rao *et al.*^[13] used CCTV for counting the annulations and found a variation of 1–2 years from actual age.

When the linear regression slope was plotted, 18 points were clustered very close to the slope, with four of them being on the slope. This further emphasizes the reliability of using cemental annulations for age determination. Miller *et al.* reported that points were scattered away from the slope in those less than 35 years of age, whereas in the > 35 years age-group, the estimated ages were clustered closer to the chronological ages. No such age-related specificity was observed in the present study.

Renz *et al.*^[14] found it immensely difficult to get reproducible counts of cemental annulations at repeated

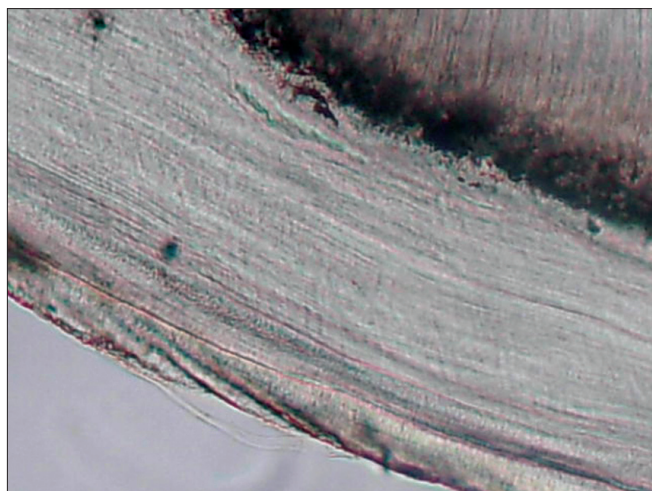


Figure 1: Photomicrograph showing cemental annulations in transverse section (Ground section, 10x digital zoom: 5x)

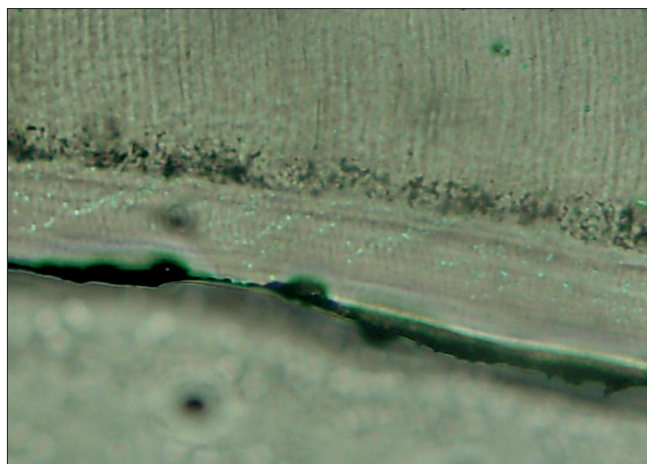


Figure 2: Indistinct and invisible annulations; longitudinal section (Ground section, 10x digital zoom: 5x)

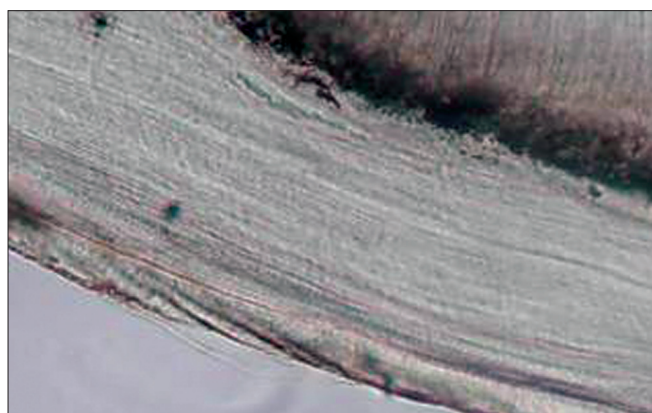


Figure 3: Clear and visible annulations transverse section (Ground section, 10x digital zoom: 5x)

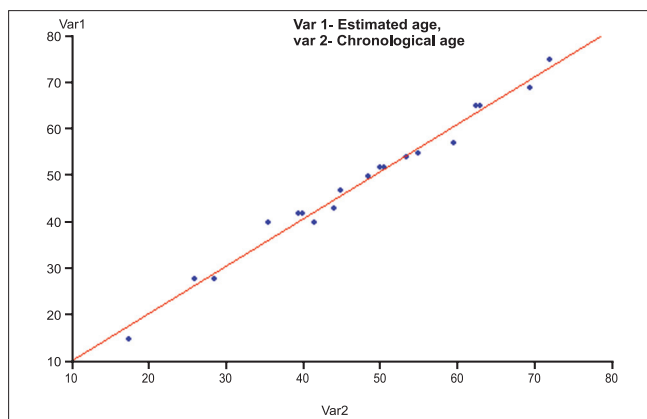


Figure 4: Linear regression slope representing the correlation between estimated age and chronological age

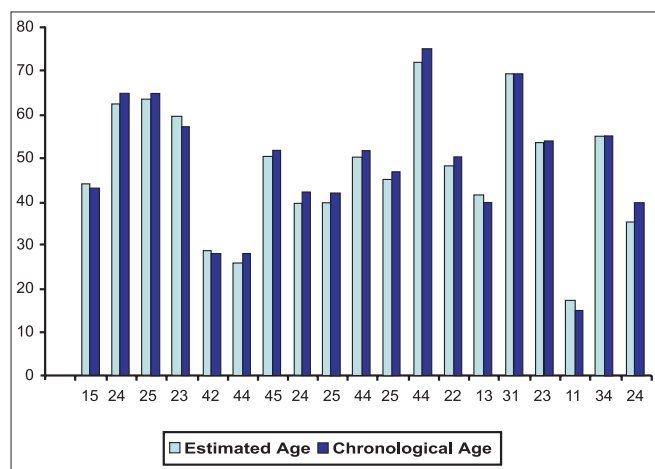


Figure 5: Bar graph of estimated age vs chronological age

counts in the same cemental area. In the present study, when counting between two observers was compared, the kappa statistic was 0.7, which indicates that there

is substantial agreement between observers in counting cemental lines.

Thus, through proper processing and sectioning, and with the use of light microscopy and photography, counting of cemental annulations can be a means of age determination.

Conclusion

Cemental annulations, when appreciated, can be used for determination of age at death of an individual. Transverse sections are better than longitudinal sections for counting annulations. There is substantial agreement between observers in counting the cemental lines.

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