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Article · December 2022

DOI: 10.53275/inapfo.2231-1092-2231-15721123

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Original Research Article

JINPAFO Vol.11, Issue 2, Jan. - June 2022

A Comparative Analysis of age Estimation of Cementum Annulations in Transverse and Longitudinal Ground Sections Using Bright Field and Phase Contrast Microscopes

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Abstract:

Introduction: Cementum is laid down in a rhythmic manner throughout life, resulting in alternating hyper- and hypo-mineralized layers. Cementochronology involves counting the incremental rings of dental cementum for age estimation. The present study was conducted to evaluate and compare the validity and reliability of age estimation by analyzing cementum annulations in the bright field- and phase contrast microscopes.

Materials and Methods: The present prospective in-vitro study was conducted on a total of 50 teeth of all classes extracted from 25 males and 25 females. Transverse (TS) and longitudinal (LS) ground sections were prepared (n=25 each). The total cementum width and number of annulations were analyzed under bright field (BFM) and phase contrast microscopes (PCM).

Results: The mean chronological age of the patients in the TS group was 31.00 years; the mean estimated age in TS under BFM was 31.53 years and under PCM was 30.83 years. Whereas, the mean age of the patients in the LS group was 48.67 years; the mean estimated age in LS under BFM was 47.04 years and under PCM was 45.28 years.

Conclusion: Cementochronology can be a method of choice in certain situations where the other methods of age estimation cannot be applied such as when many teeth are missing or when only a tooth is available. Using transverse sections and analyzing the annulations under a PCM serves to improve the reliability and accuracy of the method.

Keywords: Cementochronology; Forensic Odontology; Histomorphometry

Introduction:

Cementochronology is a histomorphometric method for age estimation using teeth in the field of Forensic Odontology. The method comprises counting the incremental rings of dental cementum analogous to the 'annular rings' present in a tree trunk. The concept was first proposed in the early 1950s wherein it was implemented for age estimation in marine animals.[1] A multitude of studies was then performed on ungulates over the years., and eventually on human teeth in 1982 by Stott et al.[2] The authors described the technique as a 'valuable link between forensic medicine, dentistry, and anthropology.'

Access this article online

Website: www.inpafo.org

DOI: 10.53275/inapfo.2231-1092-2231-15721123

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The rationale behind the concept is that cementum is laid down in a rhythmic manner throughout life, resulting in alternating hyper- and hypo-mineralized layers. The phenomenon is produced owing to the variation in the orientation and density of the hydroxyapatite crystals between adjacent layers.[3] Microscopically, these appear as

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Received : 15-07-2022
Accepted : 25-08-2022
Published: 31-12-2022

How to cite this article: Shrijha Ganesan, Sanpreet Singh Sachdev, Tabita Joy Chettiankandy, Manisha Ahire Sardar, Harsha Karwa, Jillapally Sharanya. A comparative Analysis of age Estimation of Cementum Annulations in Transverse and Longitudinal Ground Sections Using Bright Field and Phase Contrast Microscopes. J Indo Pacific Acad Forensic Odontology 2022;11(1): 10 - 14

alternating light and dark bands; each set roughly corresponding to one chronological year. Counting the number of sets in addition to the age of eruption of the respective tooth would, thus, theoretically yield the chronological age of the individual.[4]

The method has been subject to contradictory reports by various researchers and has been criticized for the commonly noted observational errors in counting the layers.[3,5,6] The probability with which a method can provide an accurate age estimation is of utmost importance in forensic odontology. A method offering an accurate estimation with high probability could play a pivotal role in certain cases and would be highly valuable in the court of law. Therefore, the relatively old but unpopular method of cementochronology was in need of improvements with respect to reliability, reproducibility, and validity.

The introduction of phase contrast microscope by Fritz Zernicke in 1934 enabled contrast-enhanced visualization of living as well as non-living objects.[7] The microscope converts phase shifts in the light passing through a transparent specimen to brightness changes in the image, while greatly amplifying the contrast between them. The enhanced contrast has been demonstrated to be useful in making the counting of cementum annulations easier.[8]

In this context, the present research was conducted to evaluate and compare the validity and reliability of age estimation by analyzing cementum annulations in the bright field- and phase contrast microscopes. Another objective of the study was to compare the same attributes between the transverse sections (TS) and longitudinal sections (LS) of the teeth.

Materials and Methods:

Collection and allotment:

The present prospective in-vitro study was conducted on a total of 50 teeth extracted from 25 males and 25 females. All the classes of teeth were included-incisors (n=8), canines (n=8), premolars (n=16), and molars (n=18).Endodontically treated teeth and those with root resorption were excluded from the study. The demographic details of the patient were recorded by a co-investigator not involved in the microscopic analysis. Every alternate tooth of a particular class was assigned to longitudinal and transverse section groups and coded accordingly: L-Longitudinal, T-Transverse, I-Incisors, C-Canine, PM-Molars, and M-Molars, followed by serial number (examples: LC-1 or TPM-4). The coded tooth was sent to the primary investigators without mentioning the chronological age of the patient while revealing only their gender and the tooth number. The extracted teeth were stored in 10% neutral buffered formalin and then washed in running tap water for five minutes. Depending on the code, corresponding transverse or longitudinal ground sections of the tooth were prepared.

Ground Section:

Transverse sections (n=25) were prepared using the middle third section of the root cervicoapically; longitudinal sections (n=25) were also prepared using the middle third of the tooth mesiodistally. The tooth was reduced in width as much as possible by an airotor followed by gradual grinding on a Carborundum stone. A general criterion was that the grinding was complete when the section appeared transparent uniformly. It was ensured that the grinding was performed in a single direction to ensure good preservation of the cemental architecture. The sections were rinsed in water, cleaned by wiping with a Muslin cloth to remove any attached debris, allowed to dry, and then mounted using DPX.

Microscopic examination:

The mounted sections were then examined under a bright field microscope(Lynx, Lawrence and Mayo India Pvt. Ltd.) as well as a phase contrast microscope (RXL-3, Radical, India). Observation and analysis of cementum annulations were performed using the 40 Xobjective (original magnification x400 times), a 5MP micro-camera, and compatible imaging software (Procam v5.0). The total cemental width and the width between two consecutive dark bands were measured under the bright field microscope (Figure 1) and then the phase contrast microscope(Figure 2).

Dental Age estimation = Eruption age of the tooth + (width between consecutive dark bands⁄total cemental width)

The measurements were performed independently by two observers and each observer performed the measurements twice, to reduce the bias in results due to inter- as well as intra-observer variability. The mean value of the two observations made by each observer (intra-observer mean) was considered followed by the mean of observations made by the two observers (inter-observer mean). The data was tabulated in a datasheet (MS Office Excel 2019, Microsoft Redmond Campus, Redmond, Washington, United States) and

subjected to further statistical analysis by Statistical package for social sciences (SPSS v 26.0, IBM).

Results:

Both the TS and the LS groups comprised 14 mandibular and 11 maxillary teeth, respectively. The TS group comprised teeth from 12 females and 13 males, while the LS group comprised teeth from 13 females and 12 males.

The mean chronological age of the patients in the TS group was 31.00 years; the mean estimated age in TS under BFM was 31.53 years and under PCM was 30.83 years.Whereas, the mean age of the patients in the LS group was 48.67 years; the mean estimated age in LS under BFM was 47.04 years and under PCM was 45.28 years.

The difference in estimated age under different microscopes for the respective types of sections is tabulated in Table 1. When considering individual parameters in the two groups, the overall results of the paired t-tests are summarized in Table 2.

Discussion:

In the present study, the mean estimated age differed from the chronological age in a range of 0.1 to a maximum of 3.3 years, irrespective of the type of section and the microscope used. This finding is in agreement with the earlier studies that suggested a similar difference of ± 2–3 years.[5,9]An overall statistically non-significant difference was noted ($p>0.05$) between the chronological age and the ages estimated in both TS and LS groups under BFM as well as PCM. This indicated that both the types of microscopes as well as the sections give reliably accurate results.

The minimum difference in mean age was noted in transverse sections observed under PCM, making this combination more suitable for estimated age. This was in accordance with findings from a recent review of studies pertaining to cementochronology.[3] There are studies that suggested a moderate or even poor correlation between the chronological age and that estimated by cementochronology.[6,10,11] In contrast to their findings, our study showed a higher positive correlation of 0.986 in the transverse section under phase contrast microscopy.

There was also a statistically non-significant difference noted for all the pairs when considering age estimation in single and multi-rooted teeth. Therefore, it can be inferred that the gender of the patient and the class of tooth do not have a significant implication in the estimation of age by the cementochronology method.

Although there was a statistically non-significant difference in the estimated age, we found that visibility of the cemental annulations and their subsequent analysis was much easier under PCM as compared to BFM. A similar observation was reported by Kaur et al., and Pundir et al. in their research.[8,12]This implies that PCM can be employed for a more efficient morphometric analysis, improving the comfort and confidence of the forensic odontologist.

The greatest advantage offered by the cementochronology method over other existing methods is that it can be performed when a single tooth is available. The other popular methods such as those based on analysis of regressive changes of the teeth or radiographs of the jaws.[13,14] This makes it useful in scenarios such as disasters, criminal cases, and in the field of paleontology, or so-called 'paleodontology'.[15,16]

Likewise, the method presents itself with certain limitations. Extracted teeth are required for the method, which may not always be possible to practically obtain in all the scenarios. Furthermore, the method is more invasive and time-consuming as compared to the radiographic methods. The preparation of ground sections requires certain equipment as a pre-requisite which may not always be easily available in the field settings.[17] Restriction of manpower and time also limit the utility of this method in actual legal or criminal cases.

Figure 1: Measured of total cementum width and the distance between two successive cementum annulations under the bright field microscope (original magnification x400)

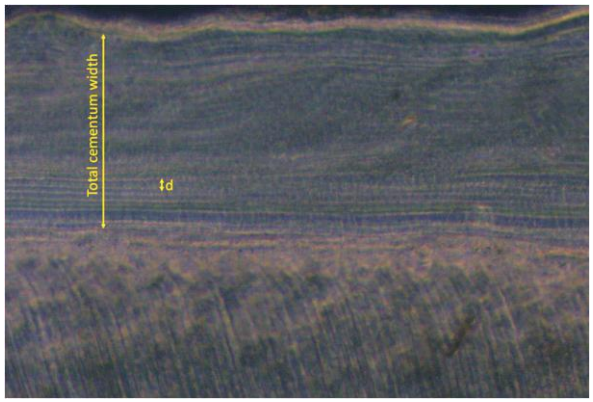

Figure 2: Improved visualization of total cementum width and the distance between two successive cementum annulations under the phase contrast microscope (original magnification x400)

Table 1: Mean differences between the chronological age and the estimated age

Transverse Sections						
Age	**Mean**	**Std. Dev.**	**Mean Difference**	**SD of diff**	**t-value**	**p-value**
CA	31.000	15.603				
BFM	31.528	16.994	Mean over-estimation by 0.528 years	3.034	-0.697	0.497
PCM	30.831	15.603	Mean under estimation by 0.168 years	4.326	0.156	0.878
Longitudinal Sections						
Age	**Mean**	**Std. Dev.**	**Mean Difference**	**SD of diff**	**t-value**	**p-value**
CA	48.670	15.603				
BFM	47.036	16.994	Mean under-estimation by 1.629 years	10.330	0.669	0.512
PCM	45.283	15.603	Mean under-estimation by 3.382 years	13.445	1.067	0.301

(CA = Chronological age; BFM = Bright Field Microscope; PCM = Phase Contrast Microscope)

Table 2: Results of paired t-tests applied across chronological age and estimated age

Transverse Sections				
Pairs	**Females**	**Males**	**Mandible**	**Maxilla**
CA and BFM	Highly significant over-estimation (p=0.004)	Non-significant	Non-significant	Significant over estimation (p=0.016)
CA and PCM	Non-significant	Non-significant	Non-significant	Significant over estimation (p=0.020)
BFM and PCM	Non-significant	Non-significant	Non-significant	Non-significant
Longitudinal Sections				
Pairs	**Females**	**Males**	**Mandible**	**Maxilla**
CA and BFM	Highly Significant underestimation (p=0.007)	Non-significant	Non-significant	Non-significant
CA and PCM	Non-significant	Non-significant	Non-significant	Non-significant
BFM and PCM	Non-significant	Non-significant	Non-significant	Non-significant

(CA = Chronological age; BFM = Bright Field Microscope; PCM = Phase Contrast Microscope)

Conclusion:

Cementochronology can be a method of choice in certain situations where the other methods of age estimation cannot be applied such as when many teeth are missing or when only a tooth is available. Using transverse sections and analyzing the annulations under a PCM serves to improve the reliability and accuracy of the method. Although its applicability may be limited due to various practical factors, it is evident that cementochronology is a reliable method for age estimation with reasonable accuracy.

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