Review Article

Age Estimation by Means of Cemental Annulations- A Review

Ramesh G, Chaubey S, Mishra G, Raj A, Seth R

Abstract: Cementum is a hard tissue in the root, which is deposited around dentin in layers. Under light microscope, root cementum is seen as alternate light and dark rings known as incremental lines. In forensic medicine numbers of these incremental lines are used to derive age at death of an individual. Since there was lack of correlation between calendar age and histological age in few studies, there was an attempt for technical improvement of the methods to study cemental annulations which were again rejected by others. Due to these discrepancies being mentioned in various studies, this paper focuses to review on which method would be the best for estimation age using cemental lines with intention that remaining difficulties can be addressed with best possible chance of success as visible distinction between different sets of annulations often appear to be vague.

Keywords: Cementum; Annulations; Tooth; Incremental lines; Microscope; Forensic odontology.

Introduction

An accurate method of age estimation is important for forensic investigators dealing with unknown bodies, parts of bodies or skeletons hard tissues of humans are able to resist decay and degradation .This can also be used for calculation of age at time of death.[1] Acellular extrinsic fiber cementum (AEFC) undergoes appositional growth resulting in even layers of alternating dark and light bands depicted from histological sections. One pair of dark and light bands each constitute one incremental line, number of which added to year of eruption of respective tooth results in histological age of individual under study.[2]

Ability to accurately estimate the ages of victims of natural or manmade disasters would be a valuable tool in forensic dentistry.[1] this technique was first described by Scheffer and Laws,[3] who observed alternating light and dark patterns in teeth of sea mammals. Stott el al,[3] introduced this technique in humans and found positive correlation between estimated and known age. To do so some authors primarily aimed at a technical improvement of the methods to study cemental annulations (CA) but others definitely rejected the methods because of lack of correlation between calendar age and histological age.[4] These contraindications are mainly due to different preparation of specimens E.g. cross-section versus longitudinal sections number and thickness of sections analyzed per tooth.[2] Due to these discrepancies being mentioned this paper focuses to review on which method would be the best for estimation age using cemental lines with intention that remaining difficulties can be addressed with best possible chance of success.[5] To perform this task literature search for articles of research studies on age estimation by counting cemental annulations done between 2000 to 2014 were searched. English literature revealed eight studies which were reviewed to evaluate whether any method in particular can be ascertained.

Discussion

Assessing the age at death, or the age of affliction of health insults during life of deceased persons, by counting tooth cementum annulations (TCAs) has shown to be cumbersome for many reasons. Optically, it is difficult to observe the annulations through the microscopy of unstained undecalcified transverse sections. Once visible, distinctions between different sets of annulations (years) often appear to be vague. Finally, some individuals seem to accumulate multiple annulations per year. Sometime a trend known as the 'doubling' phenomenon only happens during discrete periods of life.[5] Preparation of hard tissue sections is thought to be expensive and problematic to perform and also no single method has been standardized to perform counting of cemental annulations without any

problems. Hence this paper attempts to review on which is the appropriate method for age estimation by counting cemental lines and the best microscope to visualize the same.

Table 1 :Showing sample size and inclusion criteria for tooth selection.

AUTHORS	SAMPLE	INCLUSION CRITERIAN CRITERIAS
Kagerer P et al (2001)[2]	80 teeth	Pathological lesions which did no longer permit alternative treatments
Maat G et al (2006) [5]	100 Single rooted teeth	28 forensic case, 71 archaelogical cases
Aggarwal P et al(2008)[4]	30 teeth	Periodontal disease, caries, Orthodontic, Prosthetic reasons
Kaur I et al(2009)[6]	24 teeth	Freshly extracted, full mouth extraction, Orthodontic purpose, extracted from resected mandible and maxilla
Pundhir S et al (2009)[1]	40 teeth	Periodontal disease, caries, orthodontic prosthetic reasons
Avadhani A et al (2009)[7]	25 teeth	Devoid of any pathology
Dias PEM et al(2010)[3]	55 teeth	Irreversible loss of periodontal attachment, caries with periapical processes, impaction, orthodontic purpose.
Kasetty S et al (2010)[8]	200 teeth	Teeth other then molars

Table 2 : Sample cutting and types of microscopes used in various studies on cemental annulations.

AUTHOR/YEAR	Sample cutting	Type of microscope used
Kagerer P et al (2001)[2]	Longitudinal	Teeth embedded into biodur resin, Crown had been cut off from root, Phase contrast microscope
Maat G et al (2006)[5]	Transverse section Oblique transverse section	Nomarski Interference microscope
Aggarwal P et al(2008)[4]	Longitudinal	Examined under light and polarized microscope
Kaur I et al, (2009)[6]	Transverse section	Brightfield and polarized microscope, Used distilled water and quinoline for mounting to compare the visibility
Pundhir S et al (2009)[1]	Longitudinal section	Examined under light, polarized and phase contrast microscope
Avadhani A et al (2009)[7]	Half of the teeth sectioned longitudinally, Remainder were cross sectioned,	Sections visualized under a bright light microscope
Dias PEM et al (2010)[3]	Transverse sections	Observed, and photographed under optical light microscope

Kasetty S et al (2010)[8]	Longitudinal sections	One unstained section observed under polarized microscope Other section stained with alizarin red and image captured using stereomicroscope

When the articles were reviewed, several authors have used different sample size starting from 24-200 teeth[1-8], which were extracted for different reasons like periodontal disease, caries, prosthetic and orthodontic reasons.[1-4,6,8] Only Maat et al[5] had taken teeth which were from forensic (28) and archaeological cases (71) (Table 1).

Regarding the sample cutting of the specimens when reviewed, it was found that most of them used longitudinal sections.[1-3,8] Transverse sections was done by Kaur et al. (2009)[5], Dias et al. (2010)[7], where as Maat et al. (2006)[4] has done sectioning transversely and oblique transverse section i.e. perpendicular to root surface. Some authors have done sectioning on both longitudinal as well as transverse.[1,4,6,8] It was also noted that most of these authors have used more than one type of microscope to compare their results. Single type of microscope was used by many authors[2,3,5,7] and more than one type of microscope was used by others.[1,4,6,8] Most common types of microscopes used to asses cemental annulations were light microscope, polarized and phase contrast microscope.[1,2,4,7,8]In Krager et al[2] compared the cemental annulations using phase contrast and Nomarski interference microscope. In another study by Kasetty et al[8] cemental annulations was observed under polarized and stereomicroscope using two sections of same tooth, one unstained and one stained using alizarin red. Stained sections were observed under stereomicroscope (Table 2).[8]

When the comparison for the findings using various cut section of tooth and the type of microscope used was assessed, we found that, according to Aggarwal et al. (2008)[4] and Kaur et al. (2009)[6] lines were more clearly visible under polarized microscope then light microscope. Pundhir et al. (2009)[1]stated that phase contrast microscopy showed strong positive correlation between estimated and calculated age. Kasetty et al. (2010)[8] although have found positive correlation between estimated and known age when cemental annulations were counted, they have not commented on which microscope was better and have not explained findings of the stained tooth sections that was observed under stereomicroscope and its advantage (Table 3).

Table 3 : Showing the results and conclusion of various studies on cemental annulations

AUTHOR/YEAR	MATERIAL AND METHOD	RESULT
Kagerer P et al (2001)[2]	Longitudinal Phase contrast light microscope	Best results obtained in roots with fully nutritional support, precise no. of incremental lines was found in impacted teeth which never took part in mastication, worst results were obtained in category with periodontal processes.
Maat G et al (2006)[5]	Transverse section Oblique transverse section Nomarski Interference microscope	TCA layers was consistently found at marked side of all adjusted sections that had been cut perpendicular to root surface
Aggarwal P et al (2008)[4]	Longitudinal Light and polarized microscope	Lines were more clearly visible under polarized microscope then light microscope

Kaur I et al, (2009)[6]	Transverse section Brightfield and polarized microscope Used distilled water and quinoline for mounting to compare the visibility	Cemental annulations were clearly visible under polarized microscope than brightfield and the visibility of cemental annulations were enhanced under quinoline.
Pundhir S et al (2009)[1]	Longitudinal section examined under light, polarized and phase contrast microscope	Phase contrast microscopy shows strong positive correlation between estimated and calculated age
Avadhani A et al (2009)[7]	Half of the teeth sectioned longitudinally, half in transverse section, sections visualized under a bright light microscope	Transverse sections were better then longitudinal sections for counting cemental lines
Dias PEM et al (2010)[3]	Transverse sections prepared, optical light microscope	Technique was reliable for periodontally sound teeth but not for periodontally diseased teeth
Kasetty S et al (2010)[8]	Longitudinal sections prepared-One unstained section observed under polarized microscope, other section stained with alizarin red and image captured using stereomicroscope	Correlation coefficient between estimated age and known age was calculated and a positive correlation was observed

Conclusion: According to Kagerer et al (2001)[2]similar to bony tissue cementum also undergoes strict appositional growth, thus AEFC can serves as valuable aid in identification of age in subjects. According to them best results were obtained from roots with fully nutritional support precise number of incremental lines was found in impacted teeth that never took part in mastication Worst results were obtained in category with periodontal processes. Dias et al (2010)[3] emphasizes that periodontally sound teeth are better for counting cementum annulations, but Agarwal et al,(2008)[4] suggests that accuracy of TCA is independent of periodontal disease. The sectioning method to be used has also not been a topic of agreement.

Many authors prefer the sections to be longitudinal, whereas others preferred cross sections. Both methods seem to have advantages and limitations although the longitudinal sections allow viewing the whole root surface such as advocated by Klevezal and Kleinberg.[4] Maat et al (2006)[5] strongly recommended to cut the sections perpendicular to the exterior of root not perpendicular to the root axis. There is also no precisely agreed result regarding which microscope is best for viewing cementum lines for accurate age estimation. Kvaal et al, (1996)[9]emphasizes that incremental lines in cementum can also be observed in decalcified sections by using toluidine blue, cresyl violet, hematoxylin, or periodic-schiff stains by light microscopy. Explanations for this was that 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 cementum, but the results are not satisfactory.

Although most of the studies report a significantly low margin of error in age estimation under this technique, but there is a decreased accuracy of the technique in more advanced ages and the influence of periodontal diseases are still factors that require better understanding. There is also need for technical improvements and

introduction of new technologies for more accurate and appropriate results to be obtained in age estimation using cemental annulations.

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How to cite this article: Ramesh G, Chaubey S, Mishra G, Raj A, Seth R,. Age Estimation by Means of Cemental Annulations: A Review. Rama Univ J Dent Sci 2015 Mar;2(1):42-46

Sources of support: Nil **Conflict of Interest**: None declared