Efficacy Of Age Estimation In Forensic Dentistry Using Cemental Annulations As A Criteria

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Abstract

Incremental lines in tooth cementum can be used as a reliable age marker as compared to other morphological or histological traits in the human skeleton. 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 best method of studying cementum using different microscope and imbibing medias like quinoline and distilled water. One dark-light band pair was considered as one annulation which corresponds to one year of age of individual. Our results showed that incremental lines are best viewed under polarizing microscope and visibility is further enhanced by adding different imbibing media like quinoline.

INTRODUCTION

An accurate method of age estimation is important for forensic investigators dealing with unknown bodies, parts of bodies or skeletons. The best method for estimating age at death from human skeletal tissue is currently unknown. The hard tissues of human dentition are able to resist decay and degradation long after other tissues are lost. Because of this, teeth can be useful indicator of some past variation in diet or of metabolic diseases and can also be of use for calculation of age at time of death.[1]

General structural changes in teeth throughout the life are the basis for age estimation. The enamel, dentin, and cementum that comprise teeth have been used to estimate the chronological age of unidentified individuals. Because of its position cementum has not been used to the extent of enamel and dentin. However, the counting of cemental annulations may offer more accurate method for age estimation\in human beings.[2]

Zander and Hurzeler stated that cementum is potentially a better age estimating tissue due to its unique location in the alveolar process. It has been hypothesised that these incremental lines in tooth cementum can be used as a more reliable age marker than any other morphological or histological traits in the human skeleton. This hypothesis is based on the biological factors of tooth cemental annulations (TCA) known so far.[3]

The cementum consists primarily of uncalcified dense bundles of collagen fibrils. These bundles later become mineralized by hydroxyapatite crystals, whose varying orientations may be responsible for the optical effect of alternating light and dark layers.[4]

The biological explanation for the alternating layers was given by Liberman and Schroeder who suggested that dark lines are the stop phases of mineralization during the continuous growth of fibroblasts, leading to change in mineral crystal orientation. This pattern is visible under the microscope as a series of alternating light and dark lines or bands, which are known as incremental lines of cementum.[5]

The purpose of this study was to examine the correlation between age and number of incremental lines in human dental cementum under different imbibing medias and to ascertain the best method of studying cementum

MATERIALS AND METHODS

The study was carried out in Department of Oral Pathology and Microbiology, ITS-CDSR, Ghaziabd. Study groups consisted of 24 tooth specimens (6 in each group) which were categorized, the details of which are given underneath.

Group A:Freshly extracted tooth specimens from different individuals of known age.

Group B:Freshly extracted tooth specimens from full mouth extraction case of a particular

known age.

Group C:Sound tooth specimens extracted for orthodontic reasons in an individual of

known age.

Group D:Sound tooth specimens extracted from resected mandible and maxilla.

Teeth were selected and stored in 10% buffered formalin. Care was taken to preserve the integrity of root cementum after tooth extraction. Signed consent from each individual was taken for each tooth used in the individual.

The ground sections of teeth were prepared based on the method described by Slott et al.[6] The teeth were first cleaned with pumice slurry and polishing brush in Slow rotating hand piece. Tooth specimens were washed overnight with running tap water & placed in 70% alcohol. Each tooth was cut into sections using diamond tipped discs; in the middle third of the root. Middle third of the root was selected because more acellular cementum deposition is there, and hence counting of cemental annulations will be easier as it avoids hindrance by the cementocytes. Cut sections were rinsed with distilled water. Sections were dehydrated in ascending grades of alcohol (50%, 70%, 90%, 95% & 100%) for 5 min each, placed in xylene & mounted. Cementum annulations were observed in Brightfield and Polarized microscope in quinoline & distilled water media under 10X and 40X magnifications. Average of TCA counted by 3 observers was taken to avoid inter observer variability. 1 Light- dark pair was considered as 1 annulation which corresponds to 1 year age of the individual.[7] Formula used for estimation of age at death or time of tooth extraction was calculated by adding eruption age of the tooth to total number of cemental annulations.[8]

RESULTS

The study specimens showed a high correlation between the TCA and actual age of the individual as shown in Table 1. Alterations in the teeth like dental caries did not have a bearing on the validification of the formula used. The lower limit of the age range was taken for calculation and the variation range in age calculation corresponded to the age range of eruption of the tooth concerned.

Figure 1

Table 1: Comparing age estimation of different study groups

CASE No.	Tooth No.	TCA	Eruption age	Estimated age	Known age
GROUP A (Freshly Extracted Tooth specimens					
1	26	45	6.5 ± 0.5	51.5 ± 0.5	50
2	46	26	6 ± 0.5	32 ± 0.5	34
3	36	28	6 ± 0.5	34 ± 0.5	36
4	34	29	10 ± 1.0	39 ± 1.0	38
5	45	21	12 ± 1.0	33 ± 1.0	32
6	36	33	6 ± 0.5	39 ± 0.5	41
GROUP B (Full Mouth extraction cases)					
1	33	27	9.5 ± 1.0	36.5 ± 1.0	38
2	24	24	11 ± 0.5	35 ± 0.5	38
3	25	23	12 ± 0.5	35 ± 0.5	38
4	34	25	10 ± 1.0	35 ± 1.0	38
5	35	25	11 ± 1.0	36 ± 1.0	38
6	36	33	6 ± 0.5	39 ± 0.5	38
GROUP C (Orthodontically extracted teeth)					
1	24	0	11 ± 0.5	11 ± 0.5	11
2	14	0	11 ± 0.5	11 ± 0.5	11
3	34	0	10 ± 0.5	10 ± 0.5	11
4	44	0	10 ± 0.5	10 ± 0.5	11
5	24	0	11 ± 0.5	11 ± 0.5	11
6	34	0	10 ± 0.5	10 ± 0.5	11
GROUP D (Specimens extracted from resected mandible and maxilla)					
1	34	30	10 ± 1.0	40 ± 1.0	39
2	44	18	10 ± 1.0	28 ± 1.0	30
3	36	25	6.0 ± 0.5	31 ± 0.5	29
4	45	18	11 ± 1.0	29 ± 1.0	28
5	46	24	6.0 ± 0.5	30 ± 0.5	32
6	24	12	11 ± 0.5	23 ± 0.5	22

The age estimation of 6 random tooth specimens by counting the TCA were also observed under polarized microscopy in different imbibing media (distilled water and quinoline) and a stronger coherence was obtained by polarized microscopy with quinoline than distilled water and brightfield microscope respectively(Figure 1, 2). The results are tabulated in Table 2.

Figure 2

Table 2: Comparing age estimation by polarized microscope and brightfield microscope

S. No	Bright-field Microscope	Polarizing Microscope (distilled water)	Polarizing Microscope (Quinoline)	Known age
1	35± 2.0	37± 1.0	37.5± 0.5	38
2	28±1.0	31± 2.0	31.5± 0.5	32
3	32±1.0	35± 1.0	35.5± 0.5	36
4	26±2.0	31± 1.0	30.5± 0.5	30
5	30±2.0	33± 1.0	33.5± 0.5	34
6	36±2.0	39± 2.0	39.5± 1.0	40

Figure 3

Figure 1: TCA under brightfield microscope

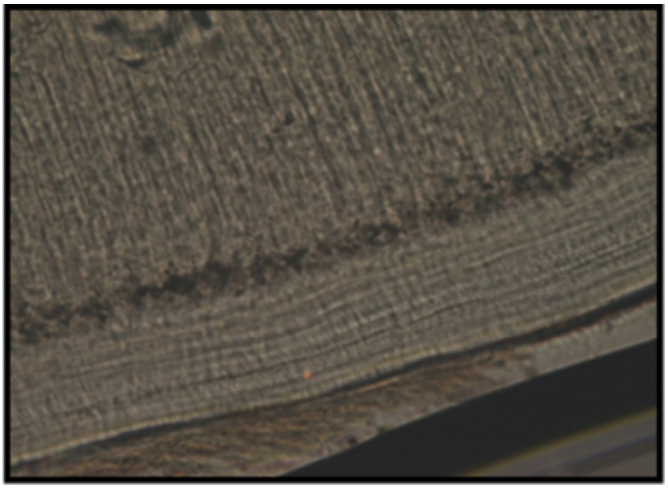
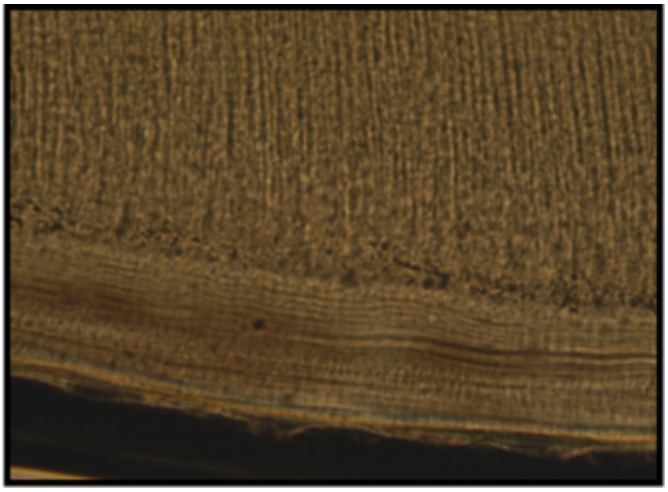


Figure 4

Figure 2: TCA under Polarized microscope imbibed in quinoline



DISCUSSION

Cementum is the calcified tissue that surrounds the root portion of dentin and forms the attachement 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.[4]

In all studies evaluating the feasibility of counting lines in human root cementum for age determination, teeth of known age were used to find a correlation between the number of root cementum lines and the age of the tooth. However, only one of these studies systematically evaluated the distribution of lines in the cementum of the middle third of the root in different sections.[9] Some studies examined different sections but only used the maximum line number for further statistical analysis and ignored the other counted line numbers.[10] Other studies took an average of different counts in different sections and areas of cementum and ‘‘ironed out’‘ the differences[11,12] Kagerer and Grupe[13] applied a method they called ‘‘sequential analysis’‘ where they tried to find the ‘‘most stable figure’‘ or the ‘‘most reliable number of layers’‘. Here too the differences were equalized. In our study we examined middle third of the root, as visibility of cemental annulations will be more pronounced in this area as it avoids hindrance by cementocytes. At apex more cellular cementum is constituted, so it obstructs the view to count cemental annulations.

The sectioning method to be used has also not been a topic of agreement. Many authors prefer the sections to be longitudinal, whereas other preferred cross sections. Both methods seems to have advantages and limitations although the longitudinal sections allow viewing the whole root surface such as advocated by Klvezal and Kleinberg, others such as Slott et al, prefer a cross section that allows a series of observations.[14] In our study we preferred cross section areas of tooth as it gave the opportunity to study series of cemental annulations.

In our study we have found that cemental annulations were more clearly visible under under polarized microscope than brightfield and hence age estimated using polarizing microscopy showed more strong correlation with sign out age of the individual. When cemental annulations were further studied under different imbibing medias like quinoline and distilled water, we found that visibility of

cemental annulations were enhanced under quinoline.

As cemental annulations are interrelated with Sharpeys fibers, it is plausible that with increasing periodontal decline, the Sharpeys fibers may lose their functional significance and decay owing to reducing alveolar bone. An arrest of cemental annulations process might occur. In our study we observed that alterations in the teeth like dental caries periodontal disease did not have a bearing on the validification of the formula used and it was supported in literature by Grosskopf et al.[15] In this study, we confirmed the visibility and countability of lines in sections from the middle third of the root.

CONCLUSION

Our results suggest that there is no statistically significant influence of sex, age, periodontal disease, or tooth type on the estimation quality of TCA method, if the described preparation and analysis standard is followed. This study demonstrates that incremental lines are best viewed under polarizing microscope and visibility is further enhanced by adding different imbibing medias like quinoline.

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