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Age estimation using annulations in root cementum of human teeth: A comparison between longitudinal and cross sections

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ABSTRACT

Background: Age estimation is an important factor in the identification of an individual in forensic science. Research indicates that cemental annulations may be used more reliably than other morphological or histological traits of human skeleton for age estimation. **Materials and Methods:** Twenty-five teeth were sectioned longitudinally, and twenty-five teeth were cross-sectioned at the mid portion of the root. Sections were ground, mounted and viewed under a bright light microscope. The area selected for counting was photographed under ×10 objective, magnified 5 times; cemental lines were counted and added to the eruption age of that patient, to obtain the chronological age. The statistical software SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data. **Results:** The *P* value comparing actual age and calculated age using longitudinal sections is moderately significant and the *P* value comparing actual age and calculated age in the age group of <30 years is significant. **Interpretation and Conclusion:** The middle third of tooth root was most suitable to count annulations. The cross sections are easier to count but longitudinal sections give more appropriate results on age estimation. Though the procedure predicts under assessment of age in the younger age group and over assessment of age in the older age group, it provides a close estimate of the actual age of an individual. It can be correlated with other age estimation methods for better reliability.
Key words: Age estimation, cemental annulations, forensic odontology

INTRODUCTION

Age is one of the essential factors in establishing the identity of the person. Estimation of the human age is a procedure adopted by anthropologists, archaeologists and forensic scientists. Different factors have been used for age estimation, but none has withstood the test of time for adults above 25 years.[1] Age estimation in cadavers, human remains and living individuals may clarify issues with significant legal and social ramifications for individuals as well as for the community.[2]

Its value and importance as an assessment tool has risen exponentially as the needs for an informed opinion on the age of an individual have assumed increasing importance for the assessment of both criminal culpability and social categorization.[3]

An accurate method of age estimation is important for forensic investigators dealing with unknown bodies, part of bodies, or skeletons. The hard tissues are able to resist decay and degradation long after other tissues are lost.[4] There is a need for a reliable method that is less sensitive to continuous age-dependent changes, in comparison with the skeleton.[5]

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Teeth can be an useful indicator of some past variation in diet or of metabolic diseases and can also be of use for calculation of age at the time of death.[4] The science dealing with establishing identity of a person by teeth is popularly known as "Forensic Odontology" or "Forensic Dentistry."[1]

Cementum is a calcified tissue that surrounds the dentine and forms the attachment site for the periodontal ligament fibers that link the tooth to the alveolar bone. It is deposited around the dentin, in layers, throughout life, thereby increasing in thickness with age.[4] Cementum is yellowish and softer than either dentine or enamel. It is made by a layer of cementum-producing cells adjacent to the dentine. The fibers of the periodontal membrane, which holds the tooth in its socket, are embedded in the cementum. Deposition of cementum continues throughout the life, especially in response to stresses. In humans, for example, as the tooth crown wears down, new cementum is deposited on the roots so that the tooth gradually rises higher in the socket and good occlusion is maintained.[6] Numerous studies have been done, wherein tooth cemental annulations have been used as a criterion for estimation of age in both land and sea animals.[7]

Cementum primarily consists 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 dark and light layers.[4] The biological explanation given for the alternating lines is that the dark lines are the stop phases of mineralization during the continued 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.[4]

Over the past 30 years, scientists have used these cemental annulations to reliably determine age of various animals.[8] Zander and Hurzeler stated that cementum is potentially a better age estimating tissue due to its unique location in the alveolar process. The hypothesis that these incremental lines in the tooth cementum can be used as a more reliable age marker than any other morphological or histological traits in the human skeleton is based on the biological factors of formation of the tooth cemental annulations. (TCA).[9]

In humans, positive association between cemental annulations and age determination has been reported by Stott *et al.*, but Lipsinic *et al.* along with Miller *et al.* have reported that cemental annulations cannot be used as a reliable age criterion. In view of these conflicting reports, the present study is an attempt to throw more light on cemental annulations and its correlation with chronological age.[8]

Historical overview

Since the early 1950's, investigations using cemental annulations for age determination have been carried out in terrestrial mammals, namely the bear, caribou, moose, elk, deer, bison, red fox, coyote, otter, squirrel and two primates – Japanese monkeys and common marmoset. The fact that these mammals, both marine and terrestrial, have cemental rings added annually to their tooth roots is well-documented. Some of the earlier investigators felt that the annulations resulted from prominent metabolic alterations induced by the prolonged fasting of the migrating seal or the extended hibernation of the bear. Later, it was discovered that countable cemental annulations corresponding to known age were found in nonhibernating animals as well. Hence, the existence and relevance of cemental annulations in numerous mammalian genera are well-documented.[7]

The first use of cementum in human age estimation began with measurements of width of the total cementum layer, rather than with counts of incremental lines (Gustafson, 1950). In the early 1980s, the study of three human teeth showed that the TCA method could be applied to human teeth as it had been to other mammals (Stott *et al.*, 1982). Further technical improvements (Naylor *et al.*, 1985) led to the suggestion that TCA is superior to other tooth-based methods of age estimation in the adult skeleton (Gustafson, 1955; Azaz *et al.*, 1974; Philipsen and Jablonski, 1992).[5]

Gustafson in 1950 suggested the use of six retrogressive changes and ranked them on arbitrary scale, allotting 0–3 points according to degree of the change. Due to error in this morphometric method, several modifications were done in subsequent studies. Johanson in 1971, in his research, used same six criteria but different ranking scale and then estimated the age of an individual. Solheim used *in situ* teeth and eight variables which included two of color estimate, two for periodontosis and two for attrition, crown length and sex. None of the changes, took separately, proved more accurate than when these were studied together.[1]

Initially, the TCA method was applied to freshly extracted teeth, but Grobkopf (1990) showed that the method was also applicable to historical skeletons and cremations. This was confirmed by others and was extended to forensic cases (Jankauskas *et al.*, 2001). These findings add further support to the idea that the number of incremental lines is a stable property, even under circumstances where other characteristics of the lines (e.g., width, degree of mineralization) have been altered by environmental or physiological perturbations (Karger and Grupe, 2001).[5]

MATERIALS AND METHODS

This study was conducted on the extracted teeth from patients of known age, visiting the Department of Oral and Maxillofacial Surgery. A sample size of 50 extracted teeth was taken up for the study. The patient case records were studied and the details were noted in the proforma to exclude pathologies such as attrition and root caries.

Inclusion criteria

Extracted teeth from patients of known age.

Exclusion criteria

- Attrition
- Hypercementosis
- Root caries
- Internal resorption
- External resorption.

Materials required

To collect the tooth specimen
- A glass specimen container
- 10% neutral buffered formalin solution.

To section the teeth
- Micromotor
- Mouth mask
- Gloves
- A diamond disc
- Lead pencil
- Rubber bowl and water.

To prepare ground sections
- Sandpaper - rough
- Sandpaper - medium
- Sandpaper - smooth
- Carborundum stone
- Xylene
- Frosted slides
- Coverslips
- DPX mountant
- Research Microscope.

Methods

A written informed consent was obtained from those undergoing extraction in the Department of Oral and Maxillofacial Surgery. The teeth included in the study were functional and devoid of any pathology such as attrition and hypercementosis. A total of 50 teeth were selected. They were preserved in formalin overnight and then washed in running water before they were sectioned using a diamond disc.

Twenty-five teeth were sectioned longitudinally so that a thin section is obtained and the remaining twenty-five teeth were transversely sectioned at the mid portion of the root [Figures 1-4]. This was followed by grinding of the sections on coarse, medium, smooth sand papers and then on the rough and smooth surfaces of the carborundum stone, in that order. These sections were immersed in xylene for 48 h for clearing and were mounted on a glass slide using DPX mountant and coverslip. Slides were allowed to dry and were

Figure 1: Image of sectioning of teeth longitudinally

Figure 2: Image of sectioning of teeth horizontally or cross sectionally

Figure 3: Grinding of teeth

then viewed under a bright light microscope. Only those teeth with cemental annulations that were suitable for counting were selected [Figures 5 and 6]. Each dark band along with the light band following it constituted one annulation. Teeth with indistinct, invisible cemental lines were eliminated. The

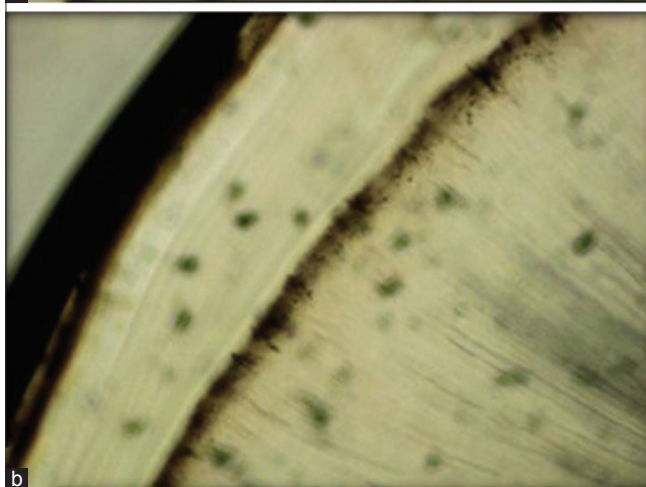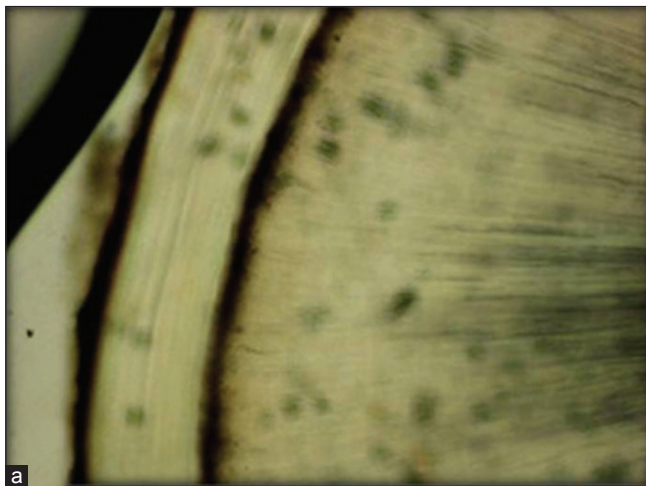**Figure 4:** Longitudinal and transverse sections of teeth

Figure 6: Photomicrograph showing cemental lines in transverse tooth section a) Ground section, x100), b) Ground section, *x*200)

Figure 5: Photomicrograph showing cemental lines in longitudinal tooth section, a) Ground section, x100), b) Ground section, *x*200)

- The number of resorption areas also increases apically
- The thinness of the cementum near the neck of the tooth inhibits scoring
- 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 area selected for counting was photographed under ×10 objective, using a digital camera and magnified 5 times. Cemental lines for each tooth were counted and added to the eruption age of that patient, to obtain the chronological age. To determine the consistency, this was checked for an intraobserver variation, wherein the same method was used and the counting was repeated after a gap of 5 days.

RESULTS AND OBSERVATION

This study included a total of 50 extracted teeth from patients of known age. Cemental annulations in longitudinal sections of 25 teeth and cross sections of 25 teeth were counted and analyzed for their correlation with the actual age of the person. In addition, an intraobserver variation

mid-root section was chosen for counting the annulations for the following reasons:
- The thickness, width and cellularity of the layers of cementum increases apically, thereby complicating the counting of annulations
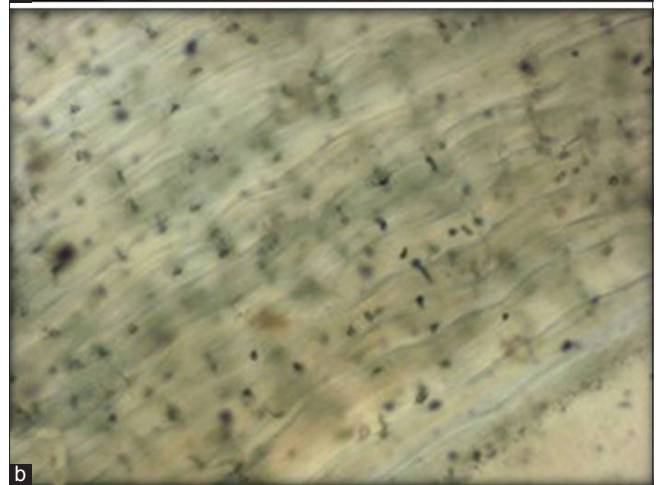
was assessed. The data obtained were subjected to statistical analysis.

Statistical methods

Descriptive and inferential statistical analyses have been carried out in the present study. Results on continuous measurements are presented on mean ± standard deviation (min-max) and results on categorical measurements are presented in number (%). Significance is assessed at 5% level of significance. The following assumptions on data made are as follows:

- Dependent variables should be normally distributed
- Samples drawn from the population should be random. Cases of the samples should be independent.

Student's *t*-test (two-tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (intergroup analysis) on metric parameters. Leven1s test for homogeneity of variance has been performed to assess the homogeneity of variance.

Statistical software

The statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables, etc.

The age distribution of patients from whom cross sections and longitudinal sections of teeth were obtained is compiled in Table 1. There were no cross sections obtained from patients <20 years (0%) and 3 longitudinal sections of teeth (10%) were obtained from patients in this group. In the age group of 21–30 years, 5 cross sections (20%) and 15 longitudinal sections of teeth (50%) were prepared. In the age group of 31–40 years, 4 cross sections (16%) and 2 longitudinal sections of teeth (6.7%) were prepared. In the age group of 41–50 years, 1 cross section (4%) and 5 longitudinal sections of teeth (16.7%) were prepared. In the age group of 51–60 years, 4 cross sections (16%) and 2 longitudinal sections of teeth (6.7%) were prepared. In the age group of 61–70 years, 10 cross sections (40%) and 2 longitudinal sections of teeth (6.7%) were prepared. In the age group of more than 70 years, one cross section (4%) and one longitudinal section of tooth (3.3%) were prepared. The mean age distribution in the cross-section group was 50.96 ± 16.88 and in the longitudinal group was 34.07 ± 16.78.

The gender distribution of the cases from whom the cross sections and longitudinal sections of teeth were obtained is represented in Table 2. Out of 25 cross section cases, 5 were male and 20 were female (25% and 75%, respectively). Out of 30 longitudinal sections, 8 were male and 22 were female (26.7% and 73.3%, respectively).

The mean count of cemental annulations in cross sections and longitudinal sections of teeth are represented in Table 3. The

Table 1: Distribution of actual age in years in cross section and longitudinal section

Age in years	Cross section *n* (%)	Longitudinal section *n* (%)
<20	0 (0.0)	3 (10.0)
21-30	5 (20.0)	15 (50.0)
31-40	4 (16.0)	2 (6.7)
41-50	1 (4.0)	5 (16.7)
51-60	4 (16.0)	2 (6.7)
61-70	10 (40.0)	2 (6.7)
>70	1 (4.0)	1 (3.3)
Total	25 (100.0)	30 (100.0)
Mean±SD	50.96±16.88	34.07±16.78

SD: Standard deviation

Table 2: Distribution of gender in cross section and longitudinal section

Gender	Cross section *n* (%)	Longitudinal section *n* (%)
Male	5 (25.0)	8 (26.7)
Female	20 (75.0)	22 (73.3)
Total	25 (100.0)	30 (100.0)

Table 3: Mean count of annulations in cross section and longitudinal section

Annulations	Cross section	Longitudinal section
First count	25.48±11.33	21.72±14.26
Second count	27.48±11.50	22.32±14.99

Table 4: Comparison of actual age and calculated age in years based on annulations

Annulations	Cross section	Longitudinal section	*P*
Actual age in years	50.96±16.88	34.07±16.78	0.001**
Calculated age on the first count	40.56±11.02	32.80±14.68	0.040*
Calculated age on the second count	42.56±10.97	33.40±15.29	0.019*
Difference			
Difference of age on the first count	10.40±19.19	2.69±14.73	0.117
Difference of age on the second count	8.40±18.26	2.08±15.03	0.186

**: Highly significant, *: Significant

Table 5: Pearson correlation of actual age and calculated age

Pairs	Cross section		Longitudinal section	
	r	*P*	*r*	*P*
Actual age versus calculated age on the first count	0.102	0.626	0.588	0.002**
Actual age versus calculated age on the second count	0.194	0.352	0.581	0.002**

**: Highly significant

mean count when the annulations were counted for the 1st time was 25.48 ± 11.33 and 21.72 ± 14.26, for cross sections and

longitudinal sections, respectively. The mean count when the annulations were counted for the 2nd time was 27.48 ± 11.50 and 22.32 ± 14.99, for cross sections and longitudinal sections, respectively.

A comparison of actual age and calculated age in years has been made between cross sections and longitudinal sections of teeth using cemental annulations and is represented in Table 4. The mean actual age in years is 50.96 ± 16.88 for cross sections and 34.07 ± 16.78 for longitudinal sections. The mean calculated age in years is 40.56 ± 11.02 for cross sections and 32.80 ± 14.68 for longitudinal sections, based on the first count. The mean calculated age in years is 42.56 ± 10.97 for cross sections and 33.40 ± 15.29 for longitudinal sections, based on the second count. The probability of chance factor (P value) is 0.001 (strongly significant), 0.040 (moderately significant) and 0.019 (moderately significant) for actual age, calculated age on the first count and calculated age on the second count, respectively. The mean difference of age on the first count is found to be 10.40 ± 19.19 for cross sections, 2.69 ± 14.73 for longitudinal sections and the $P = 0.117$ which is not significant. The mean difference of age on the second count is found to be 8.40 ± 18.26 for cross sections, 2.08 ± 15.03 for longitudinal sections and the $P = 0.186$ which is again not significant.

Pearson correlation of actual age and calculated age on the 1st and 2nd counts in cross sections and longitudinal sections is represented in Table 5. The r value of cross sections comparing actual age and calculated age on the first count and second count is 0.102 and 0.194, respectively. The P value of cross sections comparing actual age and calculated age on the first count and second count is 0.626 and 0.352, respectively, which is not significant. The r value of longitudinal sections comparing actual age and calculated age on the first count and second count is 0.588 and 0.581, respectively. The P value of longitudinal sections comparing actual age and calculated age on the first count and second count is 0.002 and 0.002, respectively, which is moderately significant.

Prediction of actual age using calculated age in years based on annulations is represented in Table 6 which is a prediction equation by regression equation.

Prediction equation by regression equation

Comparison of actual age and calculated age in years based on annulations in <30 years of age is represented in Table 7. The mean actual age in years (in <30 years) is 27.00 ± 2.82 for cross sections and 21.88 ± 2.57 for longitudinal sections. The mean calculated age in years (in <30 years) is 40.40 ± 6.38 for cross sections and 27.29 ± 14.17 for longitudinal sections, based on the first count. The mean calculated age in years (in <30 years) is 40.60 ± 5.81 for cross sections and 27.71 ± 14.14 for longitudinal sections, based on the second count. The probability of chance factor (P value) is 0.001 (strongly significant), 0.065+ (suggestive significance) and 0.068+ (suggestive significance) for actual age

Table 6: Prediction of actual age using calculated age in years based on annulations

Cross section	Longitudinal section
Actual age=44.600+0.157 × calculated age on first count R^2=1.0, P=0.626	Actual age=12.694+0.696 × calculated age on first count R^2=34.5, P=0.002**
Actual age=38.243+0.299 × calculated age on second count R^2=3.8, P=0.352	Actual age=13.422+0.660 × calculated age on second count R^2=33.8, P=0.002**

**: Highly significant

Table 7: Comparison of actual age and calculated age in years based on annulations in <30 years of age

Annulations in <30 years	Cross section	Longitudinal section	*P*
Actual age in years	27.00±2.82	21.88±2.57	0.001**
Calculated age on the first count	40.40±6.38	27.29±14.17	0.065+
Calculated age on the second count	40.60±5.81	27.71±14.14	0.068+
Difference			
Difference of age on the first count	13.40±6.30	4.79±13.65	0.197
Difference of age on the second count	13.60±5.72	5.21±13.77	0.210

**:Highly significant, +: Significant

Table 8: Comparison of actual age and calculated age in years based on annulations in>30 years of age

Annulations in >30 years	Cross section	Longitudinal section	*P*
Actual age in years	56.50±13.02	50.00±13.66	0.152
Calculated age on the first count	40.60±12.04	39.81±12.60	0.865
Calculated age on the second count	43.05±11.98	40.64±14.06	0.618
Difference			
Difference of age on the first count	16.35±16.46	12.18±10.07	0.452
Difference of age on the second count	13.90±15.97	11.36±11.28	0.645

(in <30 years), calculated age on the first count (in <30 years) and calculated age on the second count (in <30 years). The mean difference of age on the first count is found to be 13.40 ± 6.30 for cross sections, 4.79 ± 13.65 for longitudinal sections and the $P = 0.197$ which is not significant. The mean difference of age on the second count is found to be 13.60 ± 5.72 for cross sections, 5.21 ± 13.77 for longitudinal sections and the $P = 0.210$ which is again not significant.

Comparison of actual age and calculated age in years based on annulations in >30 years of age is represented in Table 8. The mean actual age in years (in >30 years) is 56.50 ± 13.02 for cross sections and 50.00 ± 13.66 for longitudinal sections. The mean calculated age in years (in >30 years) is 40.60 ± 12.04

for cross sections and 39.81 ± 12.60 for longitudinal sections, based on the first count. The mean calculated age in years (in >30 years) is 43.05 ± 11.98 for cross sections and 40.64 ± 14.06 for longitudinal sections, based on the second count. The probability of chance factor (*P* value) is 0.152 (not significant), 0.865+ (not significant) and 0.618+ (not significant) for actual age (in >30 years), calculated age on first count (in >30 years) and calculated age on the second count (in >30 years), respectively. The mean difference of age on the first count is found to be 16.35 ± 16.46 for cross sections, 12.18 ± 10.07 for longitudinal sections and the *P* = 0.452 which is not significant. The mean difference of age on the second count is found to be 13.90 ± 15.97 for cross sections, 11.36 ± 11.28 for longitudinal sections and the *P* = 0.645 which is again not significant.

Statistics

Student's t-test (two-tailed, independent)
Assumptions
Subjects are randomly assigned to one of the two groups. The distribution of the means being compared is normal with equal variances.

Test
The hypotheses for the comparison of two independent groups are:

H_o: $u_1 = u_2$ (means of the two groups are equal).

H_a: $u_1\ u_2$ (means of the two group are not equal).

The *t* is for test statistic, with $n_1 + n_2 - 2$ degrees of freedom, where n_1 and n_2 are the sample sizes for Groups 1 and 2. A low *P* value for this test (<0.05 for example) means that there is evidence to reject the null hypothesis in favor of the alternative hypothesis, or there is evidence that the difference in the two means is statistically significant.

Pretest
Test for variance assumption: A test of the equality of variance is used to test the assumption of equal variances. The test statistic is F with n_1-1 and n_2-1 degrees of freedom. *t*-test: (Two-sample assuming unequal variances) If the *P* value associated with the *t*-test is small (<0.05), there is evidence to reject the null hypothesis in favor of the alternative. In other words, there is evidence that the means are significantly different at the significance level reported by the *P* value. If the *P* value associated with the *t*-test is not small (>0.05), there is not enough evidence to reject the null hypothesis, and we conclude that there is evidence that the means are not different.

Significant figures

+ - Suggestive significance (*P* value: 0.05< *P* < 0.10)
* - Moderately significant (*P* value: 0.01< *P* ≤ 0.05)
** - Strongly significant (*P* value: *P* ≤ 0.01).

DISCUSSION

Age estimation is a subdiscipline of the forensic sciences and should be an important part of every identification process, especially when information relating to the deceased is unavailable. Dental maturity has played an important role in estimating the chronological age of individuals because of the low variability of dental indicators.[10]

Many researchers have suggested the use of cementum of teeth for determination of human chronologic age.[8] Previous studies have evaluated the feasibility of using cemental annulations in human cementum for age determination.[4] The aim of this study was to conduct further research to find out the accuracy with which cementum annulations can be used to estimate age, to compare the cemental annulations in longitudinal and cross sections of teeth and also to determine the consistency of the method at repeated counts in the same cemental area. This study was conducted on the extracted teeth from patients of known age, visiting the Department of Oral and Maxillofacial Surgery. A sample size of 50 extracted teeth was taken up for the study. Around 25 teeth were subjected to longitudinal sectioning and 25 for cross sectioning.

In our study, incremental line count was possible in 90% of cases. In studies by Jankauskas and Millet, visible cemental annulation suitable for counting was possible only in 82–86% and 71% cases, respectively.[11] In our study, we also made a comparability of feasibility of counting lines in cross sections and longitudinal sections. All 25 cross sections were assessed as all were feasible for counting and out of 25 longitudinal sections, only 20 sections were feasible for counting annulations and therefore, extra 5 longitudinal sections were made and annulations counted. Hence, 20% of longitudinal sections were not suitable for assessment. Our study is in accordance with a study by Avadhani, who also found that the cross sections were more feasible for counting, than the longitudinal sections.

In our study, we found that the middle third of tooth root was most suitable to count annulations, similar to the studies conducted by Millet and by Aggarval.[11] However, Harris and Roksandic *et al.* in their studies preferred the apical region of the root to observe and count cementum layers as they found that the cervical and middle regions of the cementum were the most difficult to record and most likely affected by diagenic processes.[12] However, we found that the midroot examination minimizes factors known to obscure annulations such as cementocytes, has adequate thickness and is minimally affected by local or systemic diseases.

The mean difference of age on the first count was found to be 10.40 ± 19.19 for cross sections which was similar to a study done by Dias PEM, who found a mean difference of 9.7 years.[13] The mean difference of age on the first count was found to be 2.69 ± 14.73 for longitudinal sections which was similar to a study done by Amandeep Singh who found

a mean difference of ±2.16 years. The mean age difference was higher when cross sections were used for age estimation indicating that longitudinal sections give more appropriate age estimation than cross sections.

The intraobserver variability is shown by a mean difference of age on the second count which was 8.40 ± 18.26 for cross sections, giving a value that was 2 years lesser than the first count. The mean difference of age on the second count was found to be 2.08 ± 15.03 for longitudinal sections, giving a value of just 0.6 years lesser than the first count. The mean age error values were found to be consistent when longitudinal sections were used for assessment than when cross sections were used, showing a better intraobserver agreement for longitudinal sections than cross sections.

Few other studies have assessed the intraobserver variation like that reported by Jankauskas, who concluded that intraobserver bias has no significant impact, similar to our study. In a study by Renz *et al*., authors found it difficult to get reproducible counts of cemental annulations at repeated counts in the same cemental area.[14] In a study conducted by Dias PEM, the repeatability between counts was considered excellent and the authors suggested that differences between counts made from the same image, by the same observer on different occasions did not have a major influence on the errors found.[13] However, the procedure is tedious, requires unwavering patience and the intraobserver variability can be kept at minimum only by a prolonged visual concentration.

Prediction of actual age using calculated age in years based on annulations showed that the calculated age of only 15% of cross sections matched the actual age of teeth on the first count and only 29% cross sections matched the actual age of teeth on the second count. The values were not consistent. The calculated age of 69% of longitudinal sections matched the actual age of teeth on the first count and 66% longitudinal sections matched the actual age of teeth on the second count. The values had a better consistency than cross sections. Not many studies have been done comparing the cross sections and longitudinal section and in our study, the *P* value was found to be strongly significant in longitudinal sections. Hence, according to our results, longitudinal sections are better to find the age of a person than cross sections. The reason we found is that the overlapping of lines is more in cross sections than longitudinal sections.

In an actual age group of <30 years, the cross sections over assessed the age, with the calculated age being 13 years more than the actual age. In the same age group of <30 years, the longitudinal sections also over assessed the age, with the calculated age being 4–5 years more than the actual age. Hence, the counting of cemental annulations for age estimation over assessed the calculated age in the younger age group of <30 years and longitudinal sections gave a better correlation than cross sections. Similar results were obtained by Miller, where, the regression analyses for the specimens in <35-year-age

group had a higher correlation coefficient and the estimated ages were clustered closer to the chronologic ages.

In an actual age group of >30 years, the cross sections under assessed the age, with the calculated age being 13–16 years less than the actual age. In the same age group of >30 years, the longitudinal sections also under assessed the age, with the calculated age being 11–12 years less than the actual age. Similar results were obtained by Miller, where, the regression analyses for the specimens in more-than-35-year-age group had a lower correlation coefficient and the estimated ages were clustered farther to the chronologic ages. Hence, the counting of cemental annulations for age estimation under assessed the calculated age in the older age group of >30 years and again the longitudinal sections gave a better correlation than cross sections. A lower correlation in older age group was also found in a study done by Lipsinic *et al* and the reasoning given is that there may be decreased apposition of cementum in individuals older than 60 years. This was further supported by Solheim, who showed that cemental apposition diminishes by one-third after the age of 60 years.[9] Furthermore, possible compression and obscuration of annulations occurs in the over-30-year-age group as a result of aging which may lead to underassessment of age in older age group.

We found that the intraobserver correlation was better in a younger age group of <30 years, with a difference of 0.2 years for cross sections and 0.42 years for longitudinal section than in an older age group of >30 years that showed a difference of 2.99 years for cross sections and 0.83 years for longitudinal sections.

In a study by Dias PEM, the difficulties faced by observers during the application of the technique, have been listed out which were similar to the difficulties that were faced by us during our study. They were variation in thickness of the LC, blurry LC on the images, overlay of the same line at different levels (that could be interpreted as two lines).[13] Structures visible in ground sections of about 100-μm thickness "disappeared" in thin sections.[15] Manual counting is time-consuming and is potentially subjective.[16] Despite these problems, the model is a cheaper, easier and more practical method and can be used as the first step before more sophisticated methods of age estimation in unknown cadavers.

Several studies have reported a straight-line relationship between age and cementum thickness. In a study conducted by Millet et al, it was concluded that the data analyzed by simple regression indicated that determining chronologic age in humans from cemental annulations is not possible.[11] In a study done by Jankauskas *et al*., it was reported that the highest correlation was found for the combined method, all correlations had a similar standard error and that the incremental lines rather have a similar use as other methods.[14] This view is also supported by a study by Willems *et al*., who suggested that instead of restricting to one particular age determination method, different techniques should be applied to establish

maximum reproducibility and to provide age estimation as reliable as possible.[10] In a study by Stein and Corcoran, it was reported that quantitation of cementum annuli alone is a moderately reliable means for age estimation in humans, similar to our opinion about using cemental annulations for age estimation.[17]

More accurate results can be obtained if the manual sections can be replaced by sections from hard tissue microtome to maintain an even thickness and to minimise anatomical changes like chipping of cementum caused by discs. The carborundum stone is of 4–5 cm diameter and grinding of teeth on this small surface was difficult. Use of hands in grinding was injurious to the fingers. Although hand grinding is tedious and injurious method, some investigators prefer this and are of the opinion that it is not replaceable by any other method.[18] Examination of all surfaces that is mesial, distal, buccal and lingual can give better values. A larger sample size can give a better correlation of cemental annulations and age. However, proper sectioning, grinding with the use of light microscopy and photography, counting of cemental annulations alone can be a means of age estimation. The longitudinal sections are better than the cross sections for estimating the age. Though there can be under assessment of age in the younger age group and over assessment of age in the older age group, this method provides a close estimate of the actual age of an individual. This method can as well be correlated with other age estimation methods for a better reliability.

CONCLUSION

Countable cemental annulations are present in human teeth. Annulations counted from a photograph or an image analyzer provides a close estimate of the actual age of the individual. The use of this method of counting cemental lines improves the accuracy of age estimation and even makes age estimation possible in cases where only poorly preserved skeletal fragments are available. Therefore, TCA age estimation by counting the LC added to the tooth's mean eruption age can be a reliable method for forensic identification and is extremely valuable in the fields of Forensic medicine, Forensic dentistry and Anthropology.

The results obtained in this study support the observations made by a plethora of previous studies that there is a relationship between cemental annulations and age of an individual. Assessment of applicability, precision and method reproducibility continue to be the focus of research in this area and are occasionally accompanied by significant controversy. Problems encountered during sectioning and potential influences on method reliability are discussed. The method can be correlated with other age estimation methods for a better reliability and documentation of similar observations with further studies will enable the use annulations in root cementum, independently in forensic age estimation.

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