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Original Article

Age estimation among North Indians by cementum annulation count: A light microscopic study

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ABSTRACT

Aim of the study: Dental hard tissues are reliable tool for age estimation. Present investigation was aimed to study cementum annulations in relation to age, sex and periodontal disease and its application in age estimation.

Material and methods: 103 ($M = 36$, $F = 67$) single rooted teeth extracted due to caries, orthodontic, periodontal disease and prosthetic reasons were collected from Department of Oral Health Sciences, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh. Labiolingual sections were prepared and cementum annulations were photographed under light microscope. Counting of annulations was done on image analysis software (VideoTesT – Size 5.0). Age was estimated by adding total number of annulations to tooth specific mean eruption age. Data was analyzed separately for pooled data, two sexes and with periodontal disease.

Results: Good correlation ($r = 0.9$) was observed between cementum annulation count and known age in pooled data as well as in two sexes (Males, $r = 0.9$ and Females, 1.0). Even, in periodontal diseased teeth, a good correlation was found ($r = 0.9$). In these cases, absolute mean error was approximately ±7.1 years as compared to ±5.7 years in teeth having no periodontal disease ($r = 1.0$). In pooled data, this error was approximately ±6.40 years.

Conclusion: It is feasible to estimate age by counting cementum annulations irrespective of sex but periodontal status of tooth has to be considered.

1. Introduction

Estimation of age is the most significant issue in the construction of profiles in living, dead and forensic cases. Many techniques are currently in use for age estimation including bone development, secondary sexual characteristics, stature height and dentition. It is easy to estimate age during the developmental stages but all the methods have their own limitations in terms of precision and need of adequate material for identification purposes. In some forensic cases, teeth are the only exceptional source for age estimation and identification of the victim. Its macroscopic, microscopic examination

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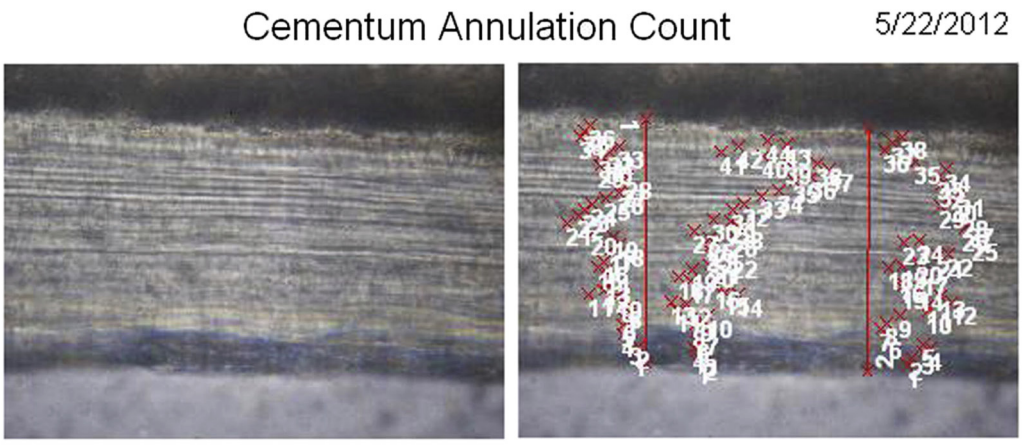

N	View	Linear1	Counter2
*		µm	number
1		687.16	-
2		683.97	-
3		-	36
4		-	44
5		-	38
✳			
	Count	2	3
	Sum	1371.13	118
	Average	685.56	39
	Std.Dev	2.25	4
	Abs.error	20.23	10
	Var.coef.	0.33	11
	Min	683.97	36
	Max	687.16	44

Fig. 1 – Tooth cementum annulations (TCA) count in upper canine, female 47 year; average no. of bands = 39; estimated age = 39 + (9 − 10) = 48–49 years.

and growth records preserved in dental hard tissues provide a means for age estimation.[1] Macroscopically age can be estimated on the basis of degree of attrition and cementum apposition but these changes can not be relied as these changes are highly influenced by lifestyle of a person.[2] Microscopically, age can be estimated on the basis of degree of degenerative processes including length of root dentine translucency, formation of secondary dentine and by counting tooth cementum annulations (TCA).

Cementum is a connective tissue that surrounds tooth roots which continues to be deposited slowly throughout life. Age can be assessed on the basis of its apposition and by counting number of annulations between cementum–dentine junction and periodontal ligament. To estimate age, one pair of dark and light band each constitute one incremental line is being added to mean eruption age of the respective tooth. This technique was originally developed for animals but was first used for humans in 1982 by Stott et al.[3] Further improvements in the technique were done by various authors which further yielded different results.[4–8] It is suggested to count incremental lines in middle 1/3rd the root because of chances of cementum resorption at cementum–enamel junction and cementum apposition at apical 1/3rd of the root.[6] Further, it has been stated that periodontal disease affects the number of incremental lines and thereby age estimation but the degree to which it affects has to be determined.[9,10]

The present investigation was designed to study cementum annulation count in relation to age, sex and periodontal disease and its utility in age estimation among North Indian population.

2. Material and methodology

For the present study, freshly extracted single rooted permanent teeth of 103 North Indians ($M = 36$, $F = 67$) were selected. The age ranges from 15 to 75 years with median age of 47 years. Samples were procured from the Department of Oral Health Sciences, PGIMER, Chandigarh. Maxillary and mandibular incisors, canines and premolars extracted for valid clinical reasons (Caries, Periodontal disease, Prosthetic and Orthodontic) were included but grossly decayed teeth that were not suitable for making sections were excluded at initial stage. Information regarding name, age, sex and details regarding periodontal status (measured with periodontal probe) was recorded before extraction. Immediately after extraction, teeth were fixed in 10% formalin for at least 24 h. After fixation, teeth were cleaned in running tap water for 24 h. For the purpose of analysis, teeth were divided into two groups: One group included teeth extracted for periodontal reasons and another group composed of teeth extracted for non-periodontal reasons (Dental caries, Prosthetic and orthodontic). The collected teeth were also divided on the basis of median age and sex of the patient.

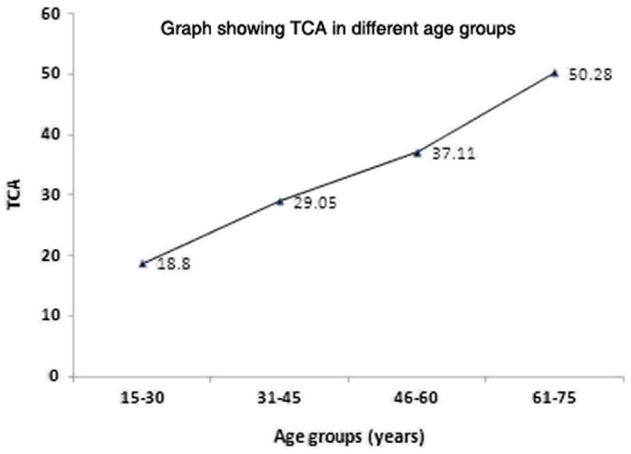

Fig. 2 – Graph showing the tooth cementum annulation count (TCA) in different age groups.

2.1. *Preparation of section*

With the help of diamond separating disc 120 μm thick labio lingually sections were prepared which were further fined by grinding up to 100 μm on carborundum stone. The thickness of the section was confirmed by digital caliper (accuracy 0.02 mm, Mitutoyo, Kanagawa, Japan). Water was used constantly as a coolant during the process of grinding that also removed debris from the section. These sections were cleaned with distilled water in ultrasonic bath for 30 s. Unstained sections were allowed to dry at room temperature and were mounted on a glass slide using DPX.

2.2. *Microscopic analysis and age estimation*

The sections were ready for microscopic analysis which was carried out in the Department of Anatomy, PGIMER, Chandigarh. The sections were examined under light microscope (Olympus CH30) attached with camera (Olympus C-7070) at 200–400× magnification. Incremental lines were identified as dark and light bands (Fig. 1); each pair of dark and light band corresponds to its one year of formation. The slide was focused at middle 1/3rd distance from the root apex. Photographs were clicked where lines were clear and running parallel to each other. The images were transferred to a computer for further analysis. These images were saved by certain codes to ensure blind study. Each observation was evaluated again after fifteen days to observe intra-observer error. For annulation count, images were imported to image analysis software (VideoTesT – Size 5.0) where the quality of these images was enhanced by adjusting contrast and color. To reduce the risk of human error each image was split into three parts. Each incremental line was marked and average was calculated from the three parts for further statistical analysis (Fig. 1). Age was estimated by adding total number of annulations to the mean eruption age of that particular tooth.

2.3. *Statistical analysis*

The data was entered and analyzed on MS Office 2007 Excel spreadsheet (Microsoft Corp. Remond, WA). A Pearson's correlation was calculated between cementum annulation count and known age. For comparison purposes, the data was subdivided on the basis of reasons for extraction, median age and sex. An independent *t*-test was used to confirm presence of significant difference between the annulation count in teeth extracted because of periodontal and non-periodontal reasons (dental caries, orthodontic and prosthetic), as well as in two sexes. A value of $p < 0.05$ was set as being statistically significant.

3. Results

Present investigation clearly showed that TCA increases with age (Fig. 2). Mean cementum annulation count in the pooled data was 33.56 ± 11.98. A strong linear correlation $r = 0.9$ was found between TCA and known age (Table 1). There were few scattered points in graph that showed a marked difference between estimated age and known age (Fig. 3). A good correlation was also found between TCA and known age in two sexes ($r = 0.9$ in males and 1.0 in females) (Table 1). No statistically significant difference was noticed in TCA count in two sexes. The difference in mean absolute error was within acceptable range in the pooled data as well as in two sexes with values of approximately ±6.40 and ±7.0 years

Table 1 – Depicting *r*, TCA count, actual age vs estimated age of pooled data, males, females, periodontal and non-periodontal diseased.

	r	Count	Actual age (years)	Estimated age (years)	Absolute mean error (years)
Whole data ($n = 103$)	0.9	33.56 ± 11.98	45.10 ± 15.62	42.35 ± 12.06	±6.40
Sex wise					
Males ($n = 36$)	0.9	33.37 ± 12.14	45.75 ± 15.88	42.65 ± 12.40	±6.70
Females ($n = 67$)	1.0	33.66 ± 11.98	44.85 ± 15.59	42.19 ± 11.97	±6.20
Periodontal status					
Periodontal teeth ($n = 54$)	0.9	33.93 ± 9.53	45.67 ± 12.87	42.59 ± 9.97	±7.1
Non-periodontal teeth ($n = 49$)	1.0	30.13 ± 13.48	39.80 ± 13.56	39.52 ± 13.56	±5.7*
Median age					
≤47 ($n = 56$)	0.9	25.49 ± 8.00	33.68 ± 10.20	34.09 ± 7.23	±5.24
>47 ($n = 47$)	0.9	43.17 ± 8.26	58.85 ± 8.05	52.19 ± 8.84	±7.78**

*$p < 0.05$ (*t*-test), **$p < 0.001$ (*t*-test).

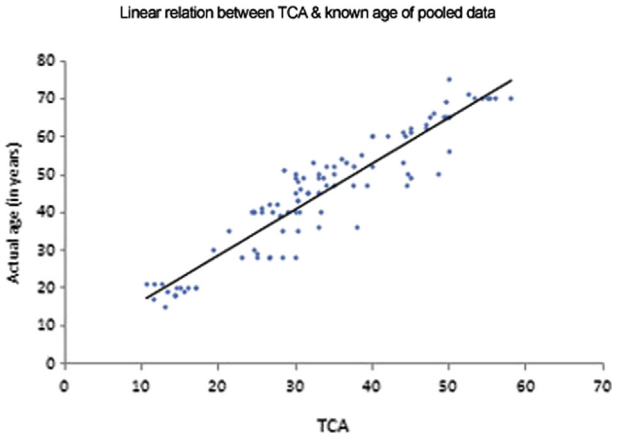

Fig. 3 – Scatter plot showing linear relation between tooth cementum annulation (TCA) count and known age of pooled data ($n = 103$).

respectively (Table 1). When data was studied on the basis of periodontal state of the tooth, correlation coefficient between TCA and known age was found to be 0.9 and 1.0 in case of teeth extracted with and without periodontal disease respectively (Fig. 4, Table 1) but statistically significant difference was noticed in TCA count of two groups. The absolute mean error was approximately ±7.1 years as compared to ±5.7 years in teeth having no periodontal disease ($p < 0.05$). Data was assessed on the basis of median age (47 years), statistically significant difference was found in absolute mean error of age between two groups ($p < 0.001$). It was comparatively smaller in younger age group (<47 years) (Table 1).

4. Discussion

Age estimation of adult human skeleton is basically a study of closure of sutures, bone development, development of secondary sexual characters but all these methods lead to determination of biological age rather than a chronological age. Application of reliable method for age estimation can help the experts to narrow their search among missing persons and identify murder or disaster victims more quickly and effectively.[11] Cementum apposition is a process that occurs throughout life in form of incremental lines thus can be proved as a promising method for age estimation, but only few studies have been reported for age estimation among North Indians.

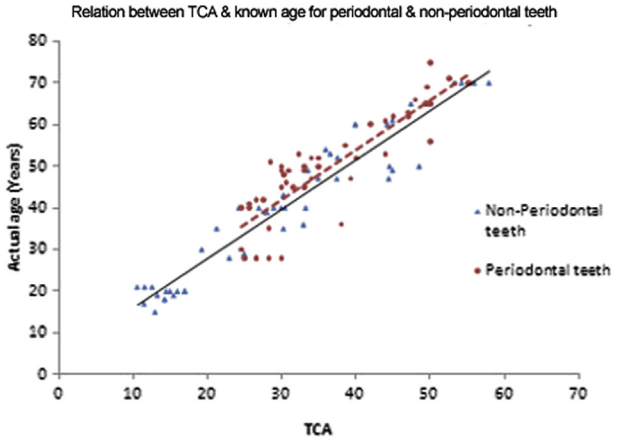

Fig. 4 – Scatter plot showing relation between tooth cementum annulation (TCA) count and known age for teeth extracted for periodontal ($n = 54$) and non-periodontal reasons ($n = 49$).

Etiology of cementum bands is unclear however, supported by theories like differences in collagen fiber orientation, degree of mineralization, seasonal variations in degree of expression.[1,12,13] Many approaches using calcified and decalcified sections have been employed to study cementum annulations.[14,15] Different stains and microscopes had also been used to enhance the readability of the same.[15,16] The present work was carried on unstained ground sections and annulations were viewed under light microscope and finally counted using computer aided software which enhanced the readability of annulations thus age estimation.

Present study (Fig. 2), showed that cementum annulation count increases with age and supports the findings of Dias et al, Aggarwal et al, Lipsinic et al.[10,16,17] Pearson correlation of 0.9 was found between cementum annulation count and known age which was in concordance with Charles et al ($r = 0.94$), Stein and Corcoran (0.93)[7,18] but higher than those observed by Dias et al ($r = 0.59$), Lipsinic et al ($r = 0.51$) and Kasetty et al ($r = 0.42$).[10,17,19]

The teeth extracted because of non-periodontal reasons ($r = 1.0$) and periodontal reasons ($r = 0.9$) showed a definite trend, with points closely grouped about the regression line (Fig. 4). Estimated ages of the teeth extracted because of periodontal disease showed that cementum annulations are severely affected by periodontal state of the tooth that may compromise precision of aging and confirm the findings of Condon et al, Dias et al, Kargerer and Groupe.[9,10,20] These findings were in contrary to the studies of Aggarwal et al. and Backofen et al.[16,21] where it was found that periodontal status seemed to have no effect on cementum annulation count. From the results of present study, it can be suggested that teeth extracted because of periodontal disease can not be grouped with another set of teeth.

When the data was analyzed on the basis of two sexes, good correlation was found between cementum annulation count and known age (Males ($r = 0.9$)) and females ($r = 1.0$) however, no significant difference was found between estimated and known age in two sexes. This suggests that there is no need to separate data on the basis of sex. The results were in concordance with similar previous works.[16,21]

An attempt was made to study cementum annulation count by dividing data on the basis of median age (47 years). Positive correlation (0.9) was found in both age groups (Table 1). In younger age group (15–47 years) mean absolute error between estimated age and known age was ±5.24 whereas it was approximately ±8 years in individuals of 48–75 years. On this basis, technique was found to be more reliable in younger individuals.

Regardless of good correlations found between cementum annulation count and known age, there are flaws in the existing technique. Authors found that technique has drawback in view of unscorable incremental lines because of faded,

overlapped or bifurcated annulations. Other researchers like Backofen et al. Avadhani et al and Miller et al[21–23] also faced the same difficulties that forced the rejection of samples at initial stages of examination.

5. Conclusion

Results of present study suggest cementum bands give reliable and acceptable data for age estimation irrespective of sex but periodontal status of the tooth has to be considered. Study further reveals that the technique is more reliable in below 47 years of age.

Conflicts of interest

All authors have none to declare.

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REFERENCES

1. Hilson S. *Dental Anthropology*. Cambridge: Cambridge, University Press; 1996.
2. Schug GR, Brandt ET, Lukacs JR. Cementum annulations, age estimation, and demographic dynamics in Mid-Holocene foragers of North India. *Homo*. 2012. http://dx.doi.org/10.1016/j.jchb.2012.01.002.
3. Stott GG, Sis RF, Levy BM. Cemental annulations as an age criterion in forensic dentistry. *J Dent Res*. 1982;61:814–817.
4. Naylor JW, Miller WG, Stokes GN, Stott GG. Cemental annulation enhancement: a technique for age determination in man. *Am J Phys Anthropol*. 1985;68:197–200.
5. Kvaal SI, Solheim T, Bjerketvedt D. Evaluation of preparation, staining and microscopic techniques for counting incremental lines in cementum of human teeth. *Biotech Histochem*. 1996;71:165–172.
6. Maat GJR, Gerretsen RRR, Aarents MJ. Improving the visibility of tooth cementum annulations by adjustment of the cutting angle of microscopic sections. *Forensic Sci Int*. 2006;159S:S95–S99.
7. Charles DK, Condon K, Cheverud JM, Buisktra JE. Estimating age at death from growth layer groups in cementum. In: Iscan, ed. *Age Markers in Human Skeleton*. Springfield, IL: Charles C Thomas; 1989:277–301.
8. Xiaohu X, Philipsen HP, Jablonski NG, Pang KM, Jiazhen Z. Age estimation from structure of adult human teeth: a review of the literature. *Forensic Sci Int*. 1992;54:23–28.
9. Condon K, Charles DK, Cheverud JM, Buikstra JE. Cementum annulations and age determination in *Homo sapiens*. II. Estimates and accuracy. *Am J Phys Anthropol*. 1986;71(3):321–330.
10. Dias PEM, Beaini TL, Melani RFH. Age estimation from dental cementum incremental lines and periodontal disease. *J Forensic Odontostomatol*. 2010;28(1):13–21.
11. Preety IA. The use of dental ageing techniques in forensic odontological practice. *J Forensic Sci*. 2003;48(5):1127–1132.
12. Lieberman DE. The biological basis of seasonal increments of dental cementum and their applications to archeological research. *J Archaeol Sci*. 1994;21:525–539.
13. Charles DK, Condon K, Cheverud JM, Bulkstra JE. Cementum annulations and age determination in *Homo sapiens*. I. Tooth variability and observer error. *Am J Phys Anthropol*. 1986;71:311–320.
14. Sousa EMD, Stott GG, Alves GB. Determination of age from cemental incremental lines for forensic dentistry. *Biotech Histochem*. 1999;74:185–193.
15. Kvaal SI, Solhein T. Incremental lines in human dental cementum in relation to age. *Eur J Oral Sci*. 1995;103:225–230.
16. Aggarwal P, Saxena S, Bansal P. Incremental lines in root cementum of human teeth: an approach to their role in age estimation using polarizing microscopy. *Indian J Dent Res*. 2008;19(4):326–330.
17. Lipsinic FE, Paunovich E, Houston DG, Robinson SF. Correlation of age and incremental lines in the cementum of human teeth. *J Forensic Sci*. 1986;31:982–989.
18. Stein TJ, Corcoran JF. Pararadicular cementum deposition as a criterion for age estimation in human beings. *Oral Surg Oral Med Oral Pathol*. 1994;77:266–270.
19. Kasetty S, Rammanohar M, Ragavendra TR. Dental cementum in age estimation: a polarized light and stereomicroscopic study. *J Forensic Sci*. May 2010;55(3):779–783.
20. Kargerer P, Groupe G. Age-at-death diagnosis and determination of life history parameters by incremental lines in human dental cementum as identification aid. *Forensic Sci Int*. 2001;118:75–82.
21. Backofen UW, Gampe J, Vaupel JW. Tooth cementum annulations for age estimation, results from a large known age validation study. *Am J Phys Anthropol*. 2004;123:119–129.
22. Avadhani A, Tupkari JV, Khambaty A, Sardar M. Cementum annulations and age determination. *J Forensic Dent Sci*. 2009;1(2):73–76.
23. Miller C, Dove S, Cottone J. Failure of use of cementum annulations in teeth to determine age in humans. *J Forensic Sci*. 1988;33(1):137–143.