DENTAL AGE ESTIMATION USING CEMENTAL ANNULATIONS: LINES TO PONDER

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ABSTRACT

Aim/Objective: No single skeletal indicator of age at death is ever likely to reflect accurately the many factors that accumulate with chronological age. Dental age estimation has proven to be important in both living and dead individuals. Cementum bands in teeth provide reliable and accurate data on the season of death of animals and their age of death. This was sought to be analysed in the current study.

Materials and Method: The study included three groups: Freshly extracted tooth specimens from different individuals, multiple extracted teeth from single case and teeth removed from corpses. Consecutive 100- to 150-micron thick sections were cut and labeled. The transverse sections were scanned in brightfield and 3 different areas having regular cemental annulations were selected, studied at both low and high power, counted and evaluated.

Result: In group A the study samples showed an estimated age value to vary from the known age of the individual by 0.5 to 1.5 yrs. Similar results were noted for Group B where, the age variation was found to be slightly higher (±3 yrs). The data analysed for group C showed minimal variation from the known age (±0.5 yrs).

Conclusion: Annulations counted from a photograph provide a close estimate of the actual age of the individual from which the tooth was extracted. This technique is reasserted to be extremely valuable in forensic medicine, forensic dentistry, and anthropology.

Keywords: cementum, incremental lines, seasonal variation, age estimation.

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INTRODUCTION

No single skeletal indicator of age at death is ever likely to reflect accurately the many factors that accumulate with chronological age. Dental age estimation has proven to be important in both living and dead individuals.

Various criteria have been used that are uniformly applicable from infancy to late adolescence (age ± 1 yr). A high inter individual variability results in error margins (minimum upto 7 years) after skeletal growth is complete. The problem intensifies at older ages, as individual variability of age-dependent changes in the skeleton increase[1]. Thus, methodological problems increase with the age of the person. It is clear then that an age estimation method is required which is less sensitive to continuous and non- quantified age dependent changes in the skeleton.

Cementum is a connective tissue that surrounds the root in incremental layers. Cementum bands in teeth provide reliable and accurate data on the season of death of animals and their age of death. Many archaeologists are aware of these incremental bands in dental cementum and have used it successfully in age estimation[2]. This has successfully been applied for age estimation in numerous animal studies also[3,4,5,6].

The age at death can be estimated by adding the number of seasons of cementum growth to the age at which the tooth erupted, and the season of death can be estimated by the nature of the outermost band[2].

Unlike other dental tissues, cementum is deposited continuously throughout life. Several microns of dense but collagen poor intermediate cementum is laid down after root completion and before the final occlusion is reached. The first deposited cementum has uncalcified collagen fibre bundles which gradually calcify by the deposition of hydroxyapatite crystals[7].

The biochemistry of the metabolism associated with this phenomenon is not known. There is some speculation regarding changes in quantities of mineral salts laid down, and/or differences in growth rates of cemental tissues at different times of the year, resulting in the appearance of concentric lines in the cementum which can be equated with years[7,8]. Variations in cementogenesis that change the appearance of lines may be induced by different factors, including, for example, biomechanical forces, nutrition, hormonal cycle, or ecological conditions such as temperature, ultraviolet light, humidity, altitude, or pollution[2,8.9].

Each pair of lines corresponds to 1 year of life and constitutes a biological record that can be used to estimate the age of an individual. Acellular cementum increments are more useful than cellular cementum for estimating the age at death of animals, since they are laid down in a comparatively regular fashion[2,7].

We present a study on these ‘lines to ponder’ in patient groups of which the age was known and this was compared to our annulation count.

MATERIALS AND METHODS

The samples comprised of extracted tooth specimens (Fig 1).

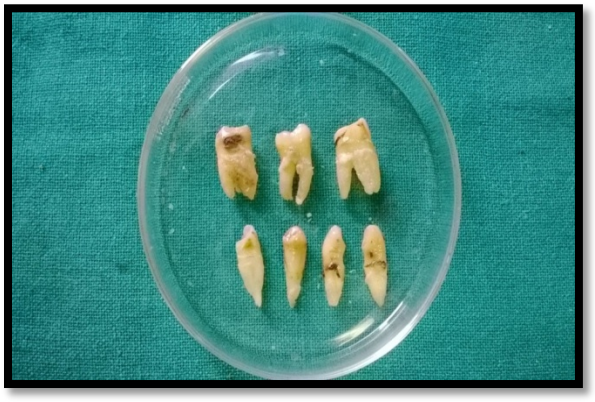


Fig 1. Photograph depicting extracted tooth specimens with intact periodontal ligament.

The study was divided into three groups:

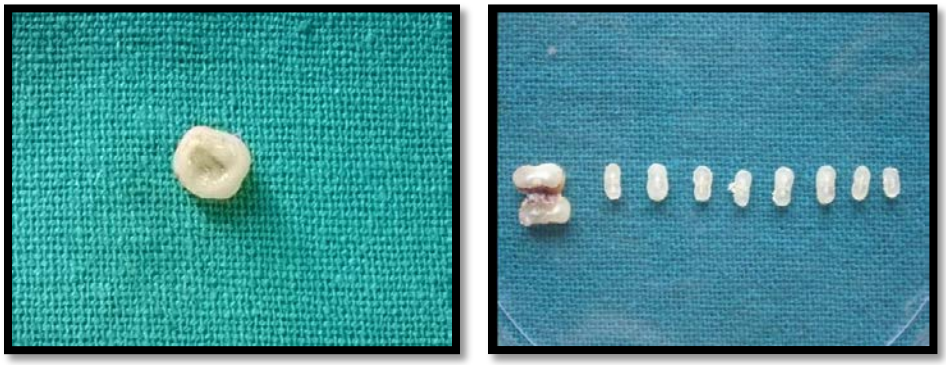
Study Groups

1. **Group A**: Freshly extracted tooth specimens from different individuals.
2. **Group B**: Multiple extracted teeth from **single** case.
3. **Group C**: Teeth removed from surgically resected jaws.

In order to maintain intact cementum the periodontal ligament was preserved while preparation of fine ground sections.

Procedure

The teeth which had been fixed in formalin were washed overnight with running tap water and placed in 70% alcohol. Teeth extracted from patients of local dentists were not suitable for study, since the cementum was always disrupted. The teeth were sectioned intact on a low speed saw using a precision diamond wafering blade. The crown of the tooth was fastened into the saw chuck, and cross-sections of the tooth were made after approximately 2 mm of the root tip were removed with the first cut. Consecutive 100- to 150-micron thick sections were cut and labeled (Fig 2).



(a) (b)

Fig 2. Photograph depicting thin transverse section prepared for the purpose of optical evaluation (a) which were further refined (b).

The transverse sections were scanned in brightfield and 3 different areas having regular cemental annulations were selected. Cementum annulations were observed in brightfield

microscope at 10x and 40x magnifications. Each light-dark pair of lines is said to constitute one annulation. For simplicity, with the large numbers of annulations involved, only the dark lines were counted.

Formula used for estimation of age at death or time of tooth extraction

Eruption age of the tooth + number of Total Cementum Annulations

RESULTS

Once the ideal areas of cementum were located and photographed, it was relatively easy to make the counts on enlarged photographs. In the current study acellular cementum was studied in the middle third of the root.

In group A the study samples showed an estimated age value to vary from the known age of the individual by 0.5 to 1.5 yrs (Table 1, Fig 3).

TABLE 1 (Group A)

S. No.	TOOTH No.	TCA	ERUPTION AGE *	ESTIMATED AGE *	ACTUAL AGE*
1.	16	49	7 ± 0.5	56 ± 0.5	56
2.	36	37	6.5 ± 0.5	43.5 ± 0.5	44
3.	13	42	9.5 ± 0.5	51.5 ± 1.0	53
4.	34	48	11 ± 1.0	59 ± 1.0	58
5.	46	24	6.5 ± 0.5	30.5 ± 0.5	31
6.	22	28	7.5 ± 1.0	35.5 ± 0.5	36
7.	45	21	12 ± 1.0	33 ± 1.0	32
8.	34	38	11 ± 1.0	49 ± 1.0	48
9.	12	39	7.5 ± 1.0	46.5 ± 0.5	47
10.	36	32	6.5 ± 0.5	38.5 ± 0.5	38

***age in years**

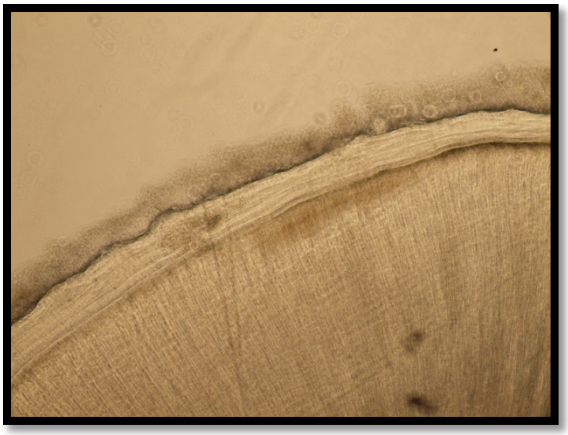


Fig 3. Photomicrograph depicting cementum annulations as viewed under brightfield.

Microscopy (Magnification X 400)

Similar results were noted for Group B where, the age variation was found to be slightly higher (±3 yrs). The findings are summarized in (Table 2, Fig 4).

TABLE 2 (Group B)

S. No.	TOOTH No.	TCA	ERUPTION AGE*	ESTIMATED AGE*	ACTUAL AGE*
1	33	39	9.5 ± 1.0	48.5 ± 1.0	48
2	25	34	12 ± 0.5	45 ± 0.5	48
3	35	33	12 ± 0.5	45 ± 0.5	48
4	14	37	11 ± 1.0	48 ± 1.0	48
5	45	35	11 ± 1.0	46 ± 1.0	48
6	36	43	6.5 ± 0.5	49.5 ± 0.5	48

***age in years**

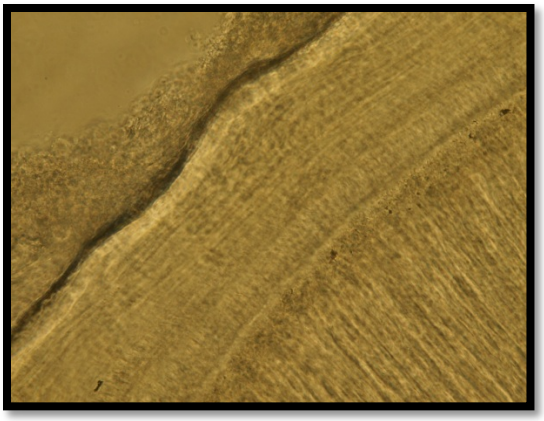


Fig 4. Photomicrograph depicting cementum annulations as viewed under brightfield.

Microscopy (Magnification X 400)

The data analysed for group C showed minimal variation from the known age (±0.5 yrs, Table 3, Fig 5).

TABLE 3 (Group C)

S. No.	TOOTH No.	TCA	ERUPTION AGE *	ESTIMATED AGE*	ACTUAL AGE*
1.	24	39	11 ± 1.0	50 ± 1.0	49
2.	34	-	11 ± 1.0	-	38
3.	23	42	9.5 ± 0.5	51.5 ± 0.5	52
4.	25	19	12 ± 1.0	30 ± 1.0	31
5.	44	14	11 ± 1.0	25 ± 1.0	26
6.	12	35	7.5 ± 1.0	43.5 ± 0.5	43
7.	15	29	12 ± 1.0	41 ± 1.0	40
8.	13	41	9.5 ± 0.5	50.5 ± 0.5	51
9.	24	12	11± 0.5	23 ± 0.5	23
10.	21	21	6.5 ± 0.5	27.5 ± 0.5	27

***age in years**

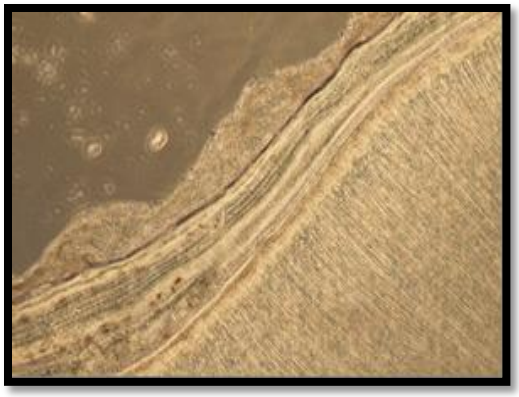


Fig 5. Photomicrograph depicting cementum annulations as viewed under brightfield.

Microscopy (Magnification X 400)

A statistical analysis of the annulation counts in different groups was done to compare the annulation counts evaluated with the known age of the specimen and the p value was estimated. It was found non- significant (p>.01).

Inference from results

1. The study specimens showed a high correlation between the TCA and actual age of the individual.
2. Alterations in the teeth like dental caries did not have a bearing on the validification of the formula used.
3. 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.
4. The cemental annulation count did not vary in presence of periodontal disease.

DISCUSSION

Cementum annulations have been studied in previous literature extensively in animals. 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 previously[2]. Since then many studies have been done to prove its usefulness in age estimation using various methods and formulae.

However, the methodology of counting cementum annulations proves useful when adequately thin sections are put to use. Through proper processing and with the correct use of light microscopy and photography, counts of cementum annulations can be a means of age determination.

It is most important that, when evaluating thick sections to remove or lower the condenser and reduce the light in order to be able to observe the elusive cementum annulations. The most common problem encountered is that the cementum lines seem to be superimposed on each other. Areas also show irregular lines. This renders counting difficult and also impossible at times. Such teeth should be removed from the study sample after scanning the microscopic image. Teeth should also be excluded if the contrast of cementum lines was too low or 'duplication' seen. These findings have been established in literature[7] and were carefully taken into consideration in the current study.

The current study supported a finding noted in literature, that the age estimation based on tooth cementum count does not vary with type and location of the tooth[10]. Also, no variation was noted on account of the tooth being from the maxillary or mandibular arch.

Inter-observer variability is another hinderance in achieving at a consensus count Such problems in the evaluation system have arisen in the past[11]. A second observer recording was also judged and the mean judged for effective age estimation.

Few authors maintain that due to various reasons, the light optical method is not satisfactory for age estimation. An ultratructural evaluation was done which enabled a better TCA count[12]. However, the methodology seems a reliable aid in age estimation, as proven in many studies[2-7,13].

The use of polarizing and phase contrast microscopic methods has also been compared to light microscopy to judge the age using cementum annulations and found successful[10,14].

From the current research study we were able to infer that the age estimation based by cementum annulation count is successful did not vary with the presence of periodontal disease (many patients had reported for extraction complaining of mobile teeth owing to loss of periodontal attachment). This finding is in congruence with that of Grosskopf et al, [15] and opposed to that of Kragerer and Grup[16,17].

A contrast study was performed recently that applied TCA to a sample of modern teeth of known demographics after experimental heat treatment at 600, 800 and 1000°C. The authors concluded that in teeth exposed to temperatures >600°C, TCA no longer yields accurate enough results to be of use in forensic investigations. Foamy fleece like remains were left after the heat treatment. This study is crucial since it puts into question the utility of tooth cementum where the subjects are burnt and mutilated in mass disasters involving fire[18].

In another recent relevant study incisors, canines, premolars, and molars, were cut and polished at progressively thin sections from archaeological specimens of unknown ages. Interobserver error indicated that viewing and counting cementum layers can prove to be a difficult process that can lead to large age ranges per individual. A high level of subjectivity exists that increases intra- and interobserver error. Chemical diagenetic processes affect the integrity of archaeological dental tissue, often obscuring and/or creating additional layers within the cementum. The researchers concluded that there are incremental layers within dental cementum that correlate positively with age but, the variables, such as subjectivity in counts and diagenetic processes, that affect the observability of cementum layers is important[19].

The present research reemphasizes the role of cementum annulations as a tool in age estimation. This has been reasserted in literature when proper technique is followed and annulations carefully counted[20,21].

In conclusion, the current study highlights the reliability and importance of the age estimation criteria using cementum annulations and confirms that the proper usage of simple light microscopy, the golden standard can also be effectively put to use. The hindrances can be countered and the obstacles confronted through proper sample handling and technique usage. Cross-sections through undecalcified tooth roots can be properly stained and mounted so that cemental annulations can be photographed through a light microscope. Annulations counted from a photograph provide a close estimate of the actual age of the individual from which the tooth was extracted. This technique may be extremely valuable in forensic medicine, forensic dentistry, and anthropology.

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