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Age Estimation by Cemetal Annulations (A Short Study)

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Abstract

Background:- Cementum (hard tissue of root), is deposited in layers around the dentin all throughout life. Root cementum is visible as alternate light and dark rings known as incremental lines of cementum under light microscopy. These annulations can be reliably used for age estimation in forensic science.

Aim & Objective:-To determine age estimation of human skeleton by cemental annulations of extracted tooth. The primary objectives of the study were to count number of cemental annulation of freshly extracted human teeth after longitudinal cross-section and to compare the results with actual age of the patients.

Materials & Method:-30teeth were selected and sectioned longitudinally. Sections were ground, mounted and viewed under a bright light microscope. The area selected for counting was photographed under 4X &10X objectives, cemental lines were counted and added to the eruption age of that patient, to obtain the chronological age.

Results:-Mean estimated age (MEA) was 45.95 years and mean actual age (MAA) was 45.42 years. The difference in the values between actual and estimated age was seen to be varying from +6 years to -3 years (approximately 9 years difference). Standard deviation was 2.77, z-value of 0.5 and p-value was 0.62 which was not significant. Correlation co-efficient showed a value of 0.98, proving strong positive correlation between the both the groups.

Conclusion:- The application of cemental annulations to determine age estimation suggestively serves as a valuable aid in forensic dentistry to identify the approximate age of an individual,

Keywords: Cemental annulations; Forensic Science; Age estimation

INTRODUCTION

Age is considered to be one of the indispensable factors, that can affect an individual's life in every aspect. Hence, it can be assumed that age estimation incorporating dental parameters can be appreciably accepted and used in human identification. However, few other genres that could gain benefit from age estimation includes- defining the legal accountability of teenagers and adults of unidentified age, support adoption procedures, helps to issue retirement funds for adults of unknown age,assist in research in archaeology and paleodemography.[1]Age estimation though dentition is commonly a dependable and unswerving method since,teeth are certainly conserved for a longer time even after all other tissues have fragmented or decomposed as teeth can endure and survive the most deleterious conditions encountered after death and during decomposition.[2,3]Cementum annulations (CA) is a microscopic technique to determine an individual's age grounded on the analysis of acellular extrinsic fiber cementum.[4,5]

Cementum being the calcified tissue surrounding the dentine, forms the attachment position for the periodontal (PDL) fibers attaching the tooth to the alveolar bone. The formation of cementum is a lifelong continuous process that is capable to increase in thickness between the ages of 20 - 60 years. This thickness differs, with maximum being at the apex and minimum near the cemento-enamel junction (CEJ). The incessant collocation of cementum may be influenced by function, occlusion, and pathologic processes.[6]These slow and regular structural changes in teeth throughout an individual's life and its unique location in the alveolar process potentially aids in age estimation. The counting of cemental annulations may suggest a more precise technique for chronological age estimation in human being.[7,8] It has been thus been conjectured that these cemental incremental lines could serve as a more dependable age marker than any other morphological or histological traits in the human skeleton. This hypothesis is dependent on the biological factors of the formation of cementum annulations (CA).[9]Cementum appears in incremental layers near the tooth roots, ensuing the formation of concentric lines (salter lines), that can be equated with years. Each pair of lines parallels to one year of life and it establishes a biological record that is helpful in age estimation.[6]

Hence, this study was undertaken with the aim to determine age estimation of human skeleton by cemental annulations of extracted tooth. The primary objectives of the study were to count number of cemental annulation of freshly extracted human teeth after longitudinal cross-section and to compare the results with actual age of the patients.

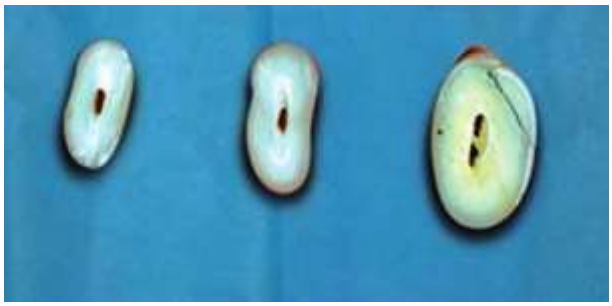
MATERIAL AND METHOD

The study was conducted in the Department of Oral Pathology & Microbiology, Kusum Devi Sunderlal Dugar Jain Dental College& Hospital, Kolkata. A total of 30 teeth were collected from the Department of Oral and Maxillofacial surgery. The teeth included in the study were functional and devoid of any pathology, tooth that needed extraction, caries free tooth. The exclusion criteria included teeth with developmental defects and also teeth of individuals with known syndrome associated with delayed eruption etc.Teeth were extracted because of periodontal disease, caries, orthodontic, and prosthetic reasons. They were preserved in 10% formalin overnight and then washed in running water before they were cross-sectioned using a diamond disc. The teeth were mounted in dental plaster. Diamond disc bur attached to a straight angled Micromotor Handpiece was used for sectioning the teeth at the middle 3rd of the root and ground sections were prepared with Arkansas stone. The sections were immersed in the Xylene for clearing for 48 hours and were mounted on slides using DPX mountant and cover slip. The slides were then viewed under light microscope – under 4X and 10X magnifications and specific areas were photographed and images were transmitted from the microscope to a computer monitor, and counting was done with the help of image analysis software. Each dark band followed by light band formed one annulation which were traced and counted by cyto-morphometric method.

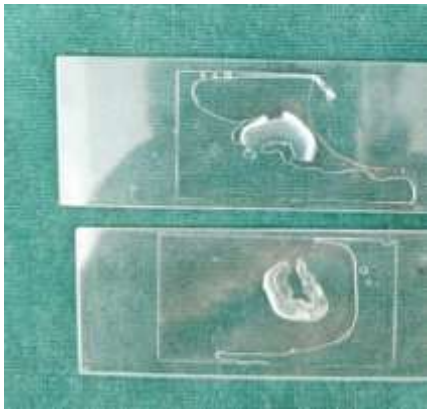
The middle 3rd portion of the root was apt for counting the annulations due to:-

- Thickness, width and cellularity cementum layers increases towards the apex, thus making it difficult to count the number of annulations.
- Thin portion of cementum at the neck of the tooth that impedes the scoring system
- Certain factors like periodontal disease or hypercementosis might make the annulations counting doubtful or might yield disparity. So, to minimize these influences the middle 3rd portion is selected.[10]


EXTRACTED TEETH SAMPLECROSS SECTIONING OF MOUNTED TEETH




CROSS – SECTIONS OF MIDROOTGRINDING OF SECTIONED SPECIMEN




MOUNTED SECTION

ANALYSISING CEMENTAL ANNULATIONS

Calculation:
The age of the patients were derived by the given formula –
No. of annulations = Width of the cementum + distance between subsequent incremental lines
Patient's age = No. of annulations + Age of eruption of that particular tooth

The final data was collected, tabulated and all the statistical analysis was performed in SPSS vs 20.01. Z-test was performed for difference of age between both set of data. Correlation co-efficient was used to see any association between the result.

RESULTS

A total of 30 freshly extracted teeth was collected and analyzed. From the sample mean estimated age (MEA) was 45.95 year and range of 23 to 92 years. Mean actual age (MAA) of patients were 45.42 years, range from 21 to 86 years. [Table 1]Largest difference of age between two sets of data was seen above the 91-100 years group and least difference was in the 61-70 years age group.[Figure 1]

The difference in the values between actual and estimated age was seen to be varying from +6 years to -3 years (approximately 9 years difference). Standard deviation was 2.77, z-value of 0.5 and p-value was 0.62 which was not significant. Correlation co-efficient after the data analysis shows a value of 0.98, proving strong positive correlation between the both the groups.[Table 2]

Table 1: Age Distribution

S.No	Age Distribution	No. of Patients	Mean Estimated Age (MEA)	Mean Actual Age (MAA)	Difference
1	21-30	6	24.50	24.56	0.06
2	31-40	5	33.53	33.37	0.16
3	41-50	4	45.74	45.61	0.13
4	51-60	6	56.13	54.81	0.32
5	61-70	1	68	68	0
6	71-80	3	76.59	75.40	1.19
7	81-90	3	86.32	83.97	2.35
8	91-100	1	92	86	6
Total		30	45.95	45.42	0.53

IMAGING

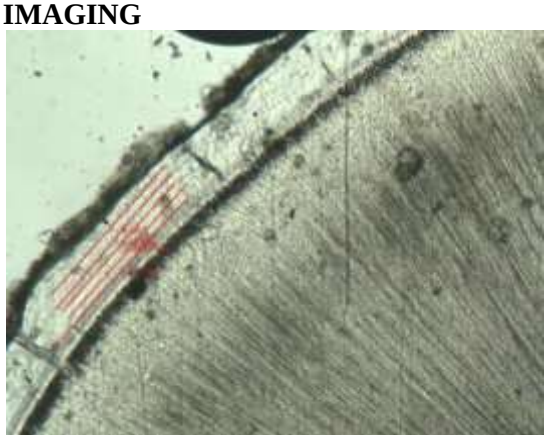


Image:1

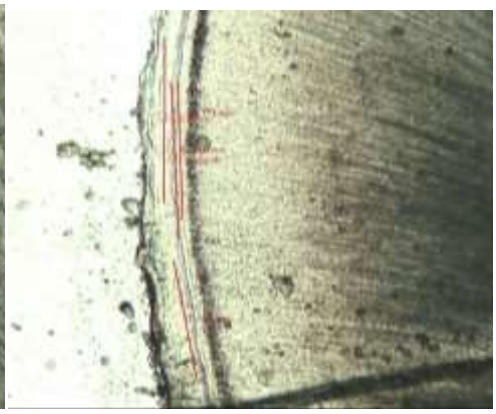


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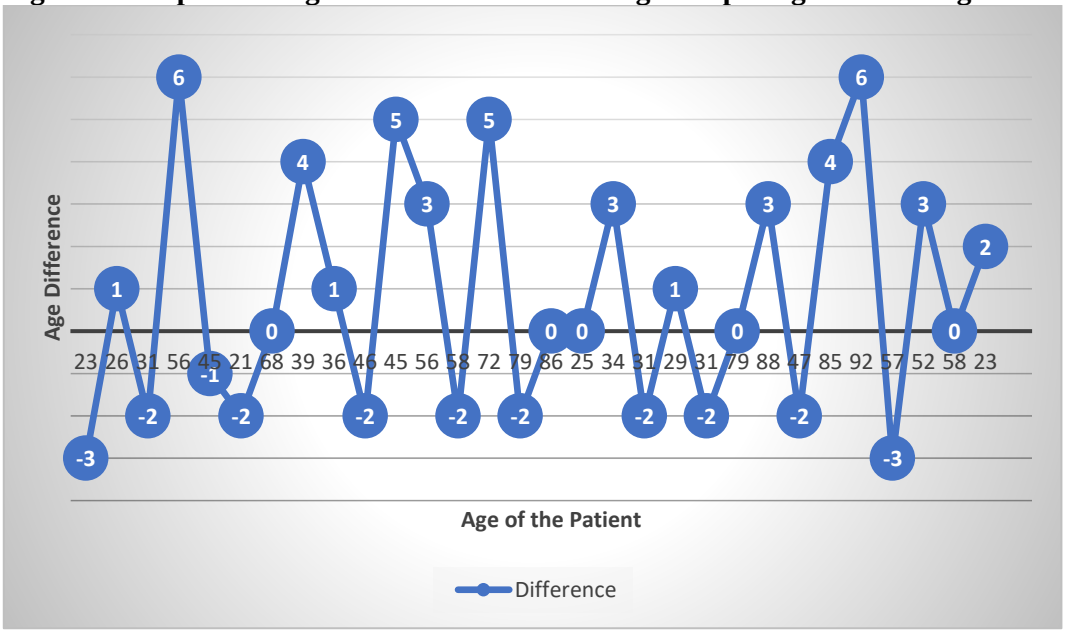
Table 2: Statistical analysis of Data

Test	Standard deviation of difference of age	Z-test	p-value	Correlation co-efficient of AA and EA
Result	**2.77**	**0.5**	**0.62**	**0.98**
Interpretation			No significant age difference between two groups	Strongly Positive Correlation

Figure 1: Graph showing Difference between the age comparing to Actual Age



DISCUSSION

Age estimation in forensic science in considered to be a helping source in order to identify an individual during a criminal activity or a mass disaster.[11]The identification of an individual (living or deceased), using the inimitable qualities and features of teeth and jaws more precisely paves an exact path in forensic odontology. Age estimation however, helps to identify the chronological age of an individual for whom the exact age is unknown more appropriately. Dental age estimation procedures are grounded on a well-regulated cascade of variations occurring during teeth development and eruption or could depend on constant progression that alter and reduce the quality of dental tissues even after the cessation of an individual's growth.[12]

Cementum is the calcified tissue surrounding the dentinal part of the root, forming the attachment site for the PDL fibers that binds the tooth to the alveolar bone. In cementum formation, there is presence of hypermineralized layers of extracellular matrix mixed with alternate less-mineralized layers. It is documented that the first layer of acellular cementum is formed prior to tooth eruption, and additional layers are added during and after eruption.[6]Incremental lines are seen in both cellular and acellular cementum.The bands in acellular cementum form and deposit at a sluggish and persistent rate as compared to that in cellular cementum. The effects of seasonal changes cause natural metabolic rhythm that could lead to changes producing layering of the cementum bands. Hormones like Parathyroid hormones along with Vitamin D helps to regulate the calcium absorption and aids in the formation of the incremental lines in layers.[11]

A biological explanation for these alternating layers was given by Lieberman and Schroder. They suggested that the dark lines were the stop phases of mineralization during continual growth of the fibroblasts, which led to a change in mineral crystal orientation. This pattern is visible under the microscope as a series of alternating light and dark lines or bands. The dark lines have been referred to as incremental lines and the cementum between each two lines has been referred to as incremental bands. It was shown that each pair of lines corresponded to one year oflife and that it constituted a biological record that could be used to estimate the age of an individual.[13]Zander and Hurzeler were the first to discover a linear relationship between the growth of cementum and chronological age based on examination of single-rooted teeth. They also observed a 3-fold increase in cemental thickness in older teeth with the highest values in the apical region.[14] Stott et al. in 1982 were the first to publish a study on the use of cementum layer counting forage estimation in humans. In their study on human cadavers they observed that counting TCAs in undecalcified, stained tooth sections provided a close estimate of actual age.[15]

Initially, the TCA method was applied to freshly extracted teeth, but Grosskopf showed that the method was also applicable to historical skeletons and cremations. However, some problems remain regarding the full application of the TCA method. For example, the small samples used in previous studies limited the establishment of statistical parameters needed for practical paleodemographic and forensic applications and the question of whether dental disease, particularly periodontal disease, has an impact on TCA.[9,16]

Hence, we aimed to determine age estimation of human skeleton by cemental annulations of extracted tooth and also compared the results with actual age of the patients. The results showed that the mean estimated age

(MEA) was 45.95 years and mean actual age (MAA) was 45.42 years. The difference in the values between actual and estimated age was seen to be varying from +6 years to -3 years (approximately 9 years difference). In a study conducted by Avadhani *et al*.,[17] the estimated age varied from chronological age by about 2-3 years. The reliability of this method was found to be 94.73 %. This was found to be different from the present study as the age variation here was seen to be approximately 9 years.

Correlation co-efficient after the data analysis from the present study, showed a value of 0.98, proving strong positive correlation between the both the groups. This was found to be in agreement with the study conducted by Aggarwal P *et al.*,[18] who showed similar results with fairly strong positive correlation between the estimated age and the actual age.

In the present study, the middle third region of the root was used for counting cementum annulations. This was because it is documented that the cementum present in the middle root region is usually acellular, uninterrupted and has an even growth that facilitates easy accountability of the annulations deprived of any interference. Lieberman and Meadow (1992) had performed a study and specified that acellular cementum have annulations that are easy to resolve microscopically since the cementum in this region is less compressed than that at the CEJ and consists of reduced cellular cementum than at the apex.[19,20,21]Huffman M *et al*. (2010) in their study had documented that the root apex was best to locate and count the cementum layers. It has been seen that the fast-growing cellular cementum at the root apex has more distinct layers as compared to the slower and thinner acellular cementum layers at the middle and cervical.[22]

It is well established that the method and type of sectioning can have an impact on the precisionand exactness of age estimation. Avadhani A *et al*. stated that transverse ground sections of teeth yield an improved result as compared to longitudinal ground sections.[17]Mallar KB *et al*. stated that cemental annulations are most aptly reckoned in the middle 3rd of the tooth root. The authors deduced that although cross-sections of tooth can be easily counted, yet more consistent results are obtained from longitudinal sections.[10] Based on all the previous literature support, we dwelled with the idea of counting the annulations through cross-sections of the teeth samples.

Microscopic examination technique could also have an influence on the accurateness of annulations counting.There are various studies involving three different methods (light,phase contrast and polarized microscopy) and comparing their accuracy, wherein it was observed thata strong positive correlation existed between the estimated age and actual age with phase-contrast microscopic technique.Pundir S *et al*.(2009) conducted a study to count the incremental lines by using light microscope, polarized microscope, and phase-contrast microscope. They performed staining of longitudinal sections of teeth by using various techniques and observed the cemental incremental lines in conventional light microscopy.Nevertheless, it did not yield a good, satisfactory result. This was due to the fact that acids and stains does not abolish the incremental lines since they contain organic structure which is different from that of the cementum.[23]Kaur P *et al*. (2015) also conducted a similar study and concluded that cemental incremental lines were clearly visible under phase contrast microscope, followed by a polarized microscope and then a light microscope.[12]

Solheim T (1992) in his study observed that in younger age group (<55 years), the annulations count seemed to be very similar and close to the actual ages whilst, those in individuals with more than 55 years of age presented an amplified propensity to be unreliable. The author also stated that cementum apposition appeared to be reduced by 1/3rd after the age of 60 years.These effects could be attributed to the decreased apposition of cementum that occurs with advancing age.[24]These findings were in concordance to the present study where it was seen that annulations were more appropriately visible in the younger age group as compared to the older ones.

Few other studies that were found to be in agreement to the present study were conducted by Singh A et al. (2013)[9], Gupta P *et al.* (2014)[6], Mallar KB *et al.* (2015)[10], Mohan N *et al.* (2018)[11]; wherein thin longitudinal ground sections were used to observe the cemental annulations under light microscope for age estimation of individuals.Hence, it was deduced from the present study that age estimation using tooth cementum annulations observed under light microscope exhibited a substantial and significant correlation between the observed age and estimated age of the individual.

It has been established from previous studies that sections from hard tissue Microtomes gives more accurate results as an even thickness of the sections is well-maintained and anatomical changes like chipping of cementum caused by discs are reduced. Use of the carborundum stone by hands in grinding is injurious to the fingers and is a tedious job, however, few investigators favor this and suggest that it is not replaceable by any other method.[25] Cemental annulations unaided can be a means of age estimation in forensic science with proper sectioning, grinding and appropriate use of light microscopy and photography.Nonetheless, there can be under and over assessment of age in the younger and older age groups respectively, this technique provides an approximate closer value correlating the estimated and actual age of an individual.[10]

There are certain limitations of cemental annulations study. This technique is not yet widely accepted by forensic specialists owing to its histological basis and it rqeuires a longer period of training and understanding of the procedure which could be exhausting and would need an expensive laboratory set-up altogether.[26]
Age estimation using cemental annulations has the probability to be used as one of the most appreciated means whose accuracy and repeatability does not rely on tooth type or location, but is attained from making as many counts as possible.

CONCLUSION

Counting cemental lines expands the exactness of age estimation which can also be carried out in cases where only poorly conserved skeletal fragments are obtainable. Thus, it can prove to be an unswerving method for forensic identification and is extremely valuable in the fields of Forensic medicine, Forensic dentistry and Anthropology. Hence, the results attained from this study was found to be in accordance to the observations made by several previous studies wherein a relationship between cemental annulations and age of an individual is well-established. However, evaluation of pertinence, meticulousness and method duplicability still remain the centre of research in this field.This can be associated with other age estimation approaches to achieve better dependability and certification of analogous observations with further studies with larger sample size.

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